FOMES ANNOSUS

Proceedings of the Third International Conference on Fomes Annosus

Aarhus, Denmark
July 29-August 3, 1968

International Union of Forest Research Organizations
Section 24: Forest Protection

Forest Service
United States Department of Agriculture, Washington, D.C.
1970

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Edited by C. S. Hodges, J. Rishbeth, and A. Yde-Andersen

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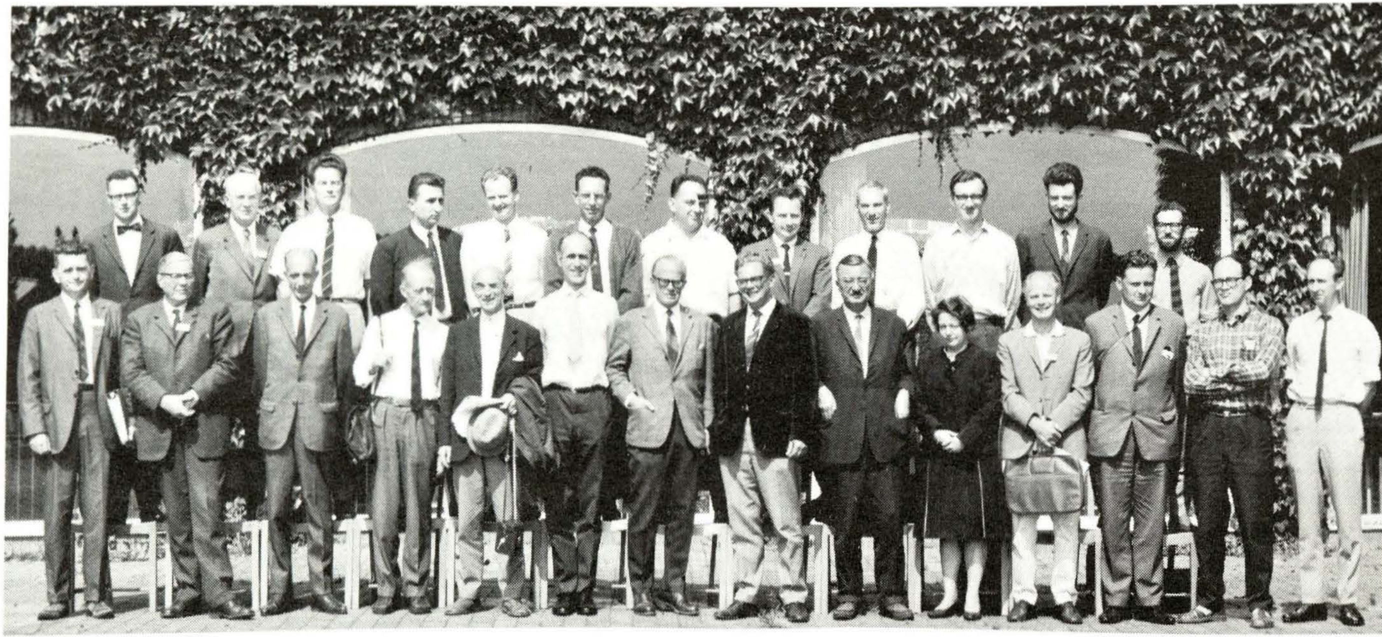
Printed by the Southeastern Forest Experiment Station,
Forest Service, USDA
Asheville, North Carolina
1970



Front Row: Left to Right

Ricard, Zycha, Laatsch, Roll-Hansen, Robak, Burdekin, Björkman, Yde-Andersen, Krstić, Aufsess, Hüppel, Laine, Shain, Pedersen.

Back Row: Left to Right

Johansson, Kramer, Kallio, Dimitri, Rehfuess, Shea, Moriondo, Cowling, Rishbeth, de Brit, Punter, Greig.

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INTRODUCTION

The Third International Conference on Fomes annosus was held on the campus of Aarhus University, Aarhus, Denmark from July 29 to August 3, 1968 under the auspices of Section 24, International Union of Forest Research Organization. The program was arranged by C. S. Hodges (U.S.A.), J. Rishbeth (U.K.) and A. Yde-Andersen (Denmark). Dr. Yde-Andersen served as host to the conference.

As with previous conferences at Wageningen in 1954 and Scotland in 1960, attendance was restricted to those actively engaged in research on Fomes annosus. Most of those invited to attend and a few who were unable to attend prepared papers which were preprinted and distributed to the delegates before the meeting. These papers, as well as a few short special papers presented during the meeting, are reproduced in full in this proceeding.

The conference was made up of a number of panel discussions. All discussion sessions were taped by Dr. W. Agtby and each panel chairman was responsible for editing the tapes and preparing summaries of the introductory remarks and discussion. Final editing was done by C. S. Hodges, J. Rishbeth and A. Yde-Andersen. These summaries are reproduced in the proceedings.

OPENING REMARKS

E. Björkman
Chairman, Section 24, IUFRO

On behalf of Section 24, Forest Protection, of IUFRO it is a great pleasure for me to welcome all of you to this conference. The conference is held under the auspicies of this section.

The First Congress of Plant Pathology in London has brought many scientists in this field together from overseas as well as from Europe and has given us an opportunity to assemble also to a small meeting on the special subject of root- and butt-rots of forest trees. The field is extremely important in many countries and therefore I am glad it has been possible for us to meet for discussion on this subject. We are especially glad to have got this opportunity in Denmark where classical investigations have been carried out in this field. It is also a great pleasure for us to meet in this friendly and hospitable country. We want to express our gratitude in advance to our hosts for their efforts, and we are convinced it will be a successful meeting.

Many interesting topics are announced that will give us a good chance to contribute to the solution of the *Fomes annosus* problem. Sometimes people say: how can you work so intensively all over the world with only one fungus. The answer is that few organisms offer so interesting and varying aspects as to life conditions, site influences, infection biology, antibiotic relationships, etc. as *Fomes annosus*.

THIRD INTERNATIONAL CONFERENCE ON *FOMES ANNOSUS*

Aarhus, July 29-August 3, 1968

PROGRAM

July 29	Afternoon	Arrival
	Evening	Dinner
		Opening: Professor, Dr. E. Björkman and Dr. E. Holmsgaard
July 30	Morning	*Infection Phenomena* Dr. J. Rishbeth, Dr. C. S. Hodges, and Dr. L. Shain
	Afternoon	*Control* Dr. C. S. Hodges, Dr. J. Rishbeth, and B. J. W. Greig
July 31	Morning	*The Spread of the Fungus* Dr. D. H. Marx, Dr. A. Yde-Andersen, Dr. J. Rishbeth, and Dr. A. Hüppel
	Afternoon	*Predisposition* Professor, Dr. W. Laatsch and Dr. K. E. Rehfuess
August 1	Morning	*Fomes annosus in Second-Rotation Stands* Dr. K. Shea, D. A. Burdekin, and Dr. A. Yde-Andersen
	Afternoon	*Excursion*
August 2	Morning	*Physiology of Fomes annosus* Dr. Ellis B. Cowling and Dr. Martin Johansson
		Interaction of Fomes annosus and Other Fungi in Stems Dr. J. Ricard and Dr. M. Krstić
	Afternoon	*Excursion*
August 3	Morning	*Summary*

WHAT TO DO WHEN EVERYTHING ELSE FAILS?

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Royal College of Forestry
Institute of Forest Botany and Pathology
Stockholm 50, Sweden

The title of this subject is given to me by the discussion chairman and gives an opportunity to remind you of two publications dealing with the possibility of using decayed wood for pulping purposes: Björkman, E., O. Samuelson, E. Ringström, T. Bergek, and E. Malm. 1949. Decay injuries in spruce forests and their importance for the production of chemical paper pulp and rayon pulp. Kungl. Skogshögskolans Skrifter 4: 1-73, and Björkman, E., L. H. Forssblad, E. Malm, S. O. Regestad, E. Ringström, and S. Rydholm. 1964. The use of decayed wood from some conifers and broadleaf trees for chemical pulping purposes. Studia Forestalia Suecica 21: 1-66.

In these publications the frequency and appearance of rot of various types in standing trees of Scots pine (_Pinus silvestris_), Norway spruce, (_Picea abies_), birch (_Betula verrucosa_ and _B_. _pubescens_), and aspen (_Populus tremula_) were reported from various parts of Sweden. Decay in wood due to a number of rot fungi was studied with respect to the effect on the production of chemical pulp of different kinds. The main purpose of the investigation was to obtain an impression of the quantities of various types of decayed wood that may be mixed with sound wood without affecting the

quality of the pulp. The investigation was centered on common rot fungi and the rots caused by them. As regards coniferous wood it was chiefly the type of decay caused by Fomes annosus, most common in southern and central Sweden, and rots occurring in northern Sweden, that were examined.

The rot damage was assigned to 4 main visual decay types: soft dark rot, soft light rot, firm dark rot, and firm light rot. Since the significance of rot damage increases with the amount of decayed wood, such wood was included with the corresponding sound wood in various proportions, namely 10, 20 and 50 percent of the various types of decay; in some cases the effect of pure rot wood (100 percent) was examined. On the basis of practical experience and random sample studies a proportion of 10 percent decayed wood for coniferous wood and 20 percent for hardwood was found to be the upper limit of what can occur in practice.

Soft dark and soft light rot proved to result in a great increase in wood consumption and impairment of the quality of the pulp. When bleaching of the pulp takes place, such rot may, however, be admitted. A volume deduction of 100 percent for the damaged volume of such decay is suggested. Firm dark rot, apart from its impairment of quality, also gave a lower yield than the corresponding sound wood. A certain proportion might therefore be included in pulp wood provided that some deduction is allowed for. A deduction of 100 percent for the volume of decayed wood seems to be unnecessarily high but might be justified by the need for the maximum simplification of the assessment in practice. Firm light rot did not cause an appreciable impairment of the pulp, whatever proportion was included. Although there was undoubtedly some initial breakdown and loss of strength, this type of decayed wood may conveniently be included without deduction and thus counted as a "tolerance defect". Some compensation for the too great deduction for firm dark rot is thereby obtained. This system, which was originally recommended on the basis of the study on Fomes annosus-rot in spruce, has been applied in the instructions for scaling of pulp wood since 1948 with no impairment of the quality of the pulp. In broadleaf trees this type of rot rarely develops. Such rot damage often begins as a darkening of the wood, with gradual progress to the final stage of soft light rot. A discoloration of the wood need not be due primarily to an attack of rot and may, for the lighter forms at least, be assessed as a "tolerance defect".

ZUM ABBAU MANGANHALTIGEN FICHTENHOLZES DURCH _FOMES ANNOSUS_ (FR.) COOKE

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EINLEITUNG

Mangan ist als Mikronährstoff für das Gedeihen der Pflanzen notwendig. Bei Untersuchungen über den Manganhaushalt von Waldbäumen wurde festgestellt, dass über den eigentlichen Bedarf hinaus aufgenommenes Mangan auf das Wachstum keinen Einfluss hat. Das mit dem Transpirationsstrom in die Bäume gelangte überflüssige Mangan wird bei der Fichte besonders in Nadeln und Rinde aber auch im Holz abgelagert (Baumeister, 1954; Gonser, 1960).

Durch vorliegende Untersuchung sollte festgestellt werden, ob und in welchem Masse der enzymatische Abbau des Fichtenholzes durch _Fomes annosus_ (Fr.) Cooke vom Mangangehalt des Holzes beeinflusst wird. Ferner wollten wir Aufschluss darüber erhalten, ob Splint und Reifholz hierbei modifizierend wirken.

[1/] Unter Mitwirkung der Herren Dr. Evers, Dr. Gonser und Dr. Schlenker der Baden-Württembergischen Forstlichen Versuchs- und Forschungsanstalt, Abt. Botanik und Standortskunde, Stuttgart-Weilimdorf, BR Deutschland.

MATERIAL UND METHODEN

Untersucht wurde 55-60 jähriges Fichtenholz der Standorte Gutenzell und Illertissen mit je einer Stammscheibe langsam gewachsenen Holzes (schmälere Jahrringe) und schneller gewachsenen Holzes (breitere Jahrringe) aus 1,30 m. Stammhöhe. Beide Standorte sind hinsichtlich des festgestellten Gesamtmangans etwa gleichwertig. Der Gehalt an aktivem, pflanzenverfügbarem Mangan--nach Scheffer und Schachtschabel 1960 als Summe des leicht reduzierbaren und austauschbaren Mangans zu verstehen--war in Illertissen deutlich niedriger. Der Boden dieses Standorts ist reich an Nährstoffen. Bei einem hohen Calzium-Angebot liegen die pH-Werte hier im alkalischen Bereich. Demgegenüber ist der Gutenzeller Standort durch einen armen Nährstoffhaushalt und niedrigere pH-Werte gekennzeichnet (Tabelle 1). Die unterschiedlichen Standortverhältnisse kommen darüber hinaus nicht nur im deutlich differenzierten Mangangehalt der Fichtennadeln zum Ausdruck, sondern spiegeln sich auch im unterschiedlichen Mangananteil des Holzes wider. Die Manganbestimmungen führte Herr Dr. Evers nach Kurmies 1955 durch. Im Gutenzeller Fichtenholz wurden 22,5-50,0 mg. Mn(II) pro 100 g. Trockensubstanz gefunden, während im Holz von Illertissen nur Spruen bzw. 5,0 mg. Mn(II) nachgewiesen werden konnten (Tabelle 2).

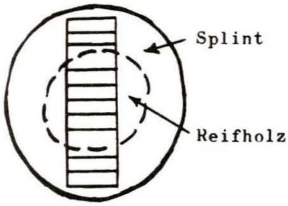
Tabelle 1

Standort	Zusammenfassende Kennzeichnung des		Gehalt der Fichtennadeln an
	Nährstoffhaushalts	Aziditätsbereichs	Mangan
Gutenzell	arm	sauer	wie Makronährstoffe Maximum: 361 mg./100 g. Trockensubstanz
Illertissen	reicher	alkalisch	wie Mikronährstoffe <7 mg./100 g. Trokkensubstanz

Tabelle 2.--Mn(II)-gehalt des Fichtenholzes pro 100 g. Trockensubstanz

Standort	Jahrringe	Reifholz mg.	Splint mg.
Gutenzell Mn-reich	breit	22,5	22,5
	schmal	50,0	45,0
Illertissen Mn-arm	breit	Spuren	5,0
	schmal	Spuren	Spuren

Aus dem Splint und Reifholz der insgesamt 4 Stammscheiben formten wir



je 10 Proben von 1 x 1 x 2,5 cm. Grösse und bestimmten das Darrgewicht bei 103° C. Die trokkenen Proben wurden mit abgekochtem Leitungswasser wieder befeuchtet und unter sterilen Bedingungen auf Malzagarplatten gelegt, die von Fomes annosus bewachsen waren. Die Inkubation betrug 15 Wochen bei 24° C. und 80% ± 5% relative Luftfeuchtigkeit. Nach Ablauf dieser Zeit wurden die ausgebauten Proben vorsichtig vom Mycel befreit, gedarrt und zurückgewogen.

ERGEBNISSE

Sämtliche Proben waren nach wenigen Tagen von mittelkräftigem Mycel des Fomes annosus gut überzogen. Im Wachstum und Habitus des Pilzes konnten wir keine Unterschiede zwischen manganreichen und manganarmen Proben bemerken.

Nach 15 Wochen betrug der mittlere Gewichtsverlust der manganarmen Proben 7,1-9,6% und der manganreichen Proben 11,9-13,9%. Letztere weisen somit im Splint und im Reifholz gegenüber den manganarmen Proben wesentlich höhere Abbauverluste auf. Die Unterschiede der vergleichbaren Proben sind mit einer Irrtumswahrscheinlichkeit von 0,1% bzw. 1,0% statistisch gesichert (Tabelle 3). Die Ergebnisse sind in Säulendiagrammen mit aufgetragener Streubreite dargestellt (Bild 1).

Um den Einfluss des Splints auf die Abbauleistung des Fomes annosus zu prüfen, wurden seine Gewichtsverluste mit denen des Reifholzes innerhalb der Standorte verglichen. Hierbei zeigte es sich, dass bei den breitringigen Proben die beobachteten Unterschiede nur geringfügig sind.

ZUSAMMENFASSUNG

1. Um den Einfluss von Mangan auf den Stoffwechsel von Fomes annosus zu prüfen, wurde Fichten Reif- und Splintholz mit unterschiedlichem, natürlichem Mangangehalt untersucht. Als Kriterium der Beurteilung diente die enzymatische Abbauleistung des Pilzes.

2. Die Splint- und Reifholzproben mit dem grösseren Mangangehalt zeigten nach der Inkubation einen höheren Gewichtsverlust als die Proben mit niedrigerem Mangangehalt. Die Unterschiede sind signifikant. Der Stoffwechsel von Fomes annosus wird offenbar durch einen höheren Mangangehalt des Holzes gefördert.

Tabelle 3

		Jahrringe	Fichte-Reifholz Mn-arm breit	Fichte-Reifholz Mn-arm schmal	Fichte-Reifholz Mn-reich breit	Fichte-Reifholz Mn-reich schmal	Fichte-Splint Mn-arm breit	Fichte-Splint Mn-arm schmal	Fichte-Splint Mn-reich breit	Fichte-Splint Mn-reich schmal	Vergleich zwischen
Fichte-Reifholz	Mn-arm	breit		-	+++		-				
		schmal				+++		+++			
	Mn-reich	breit				-			-		Splint u. Reifh.
		schmal								+	
Fichte-Splint	Mn-arm	breit						-	+++		Mn-arm u. Mn-reich
		schmal								++	
	Mn-reich	breit								++	
		schmal									

nicht gesichert - gesichert zu 99% ++
gesichert zu 95% + gesichert zu 99,9% +++

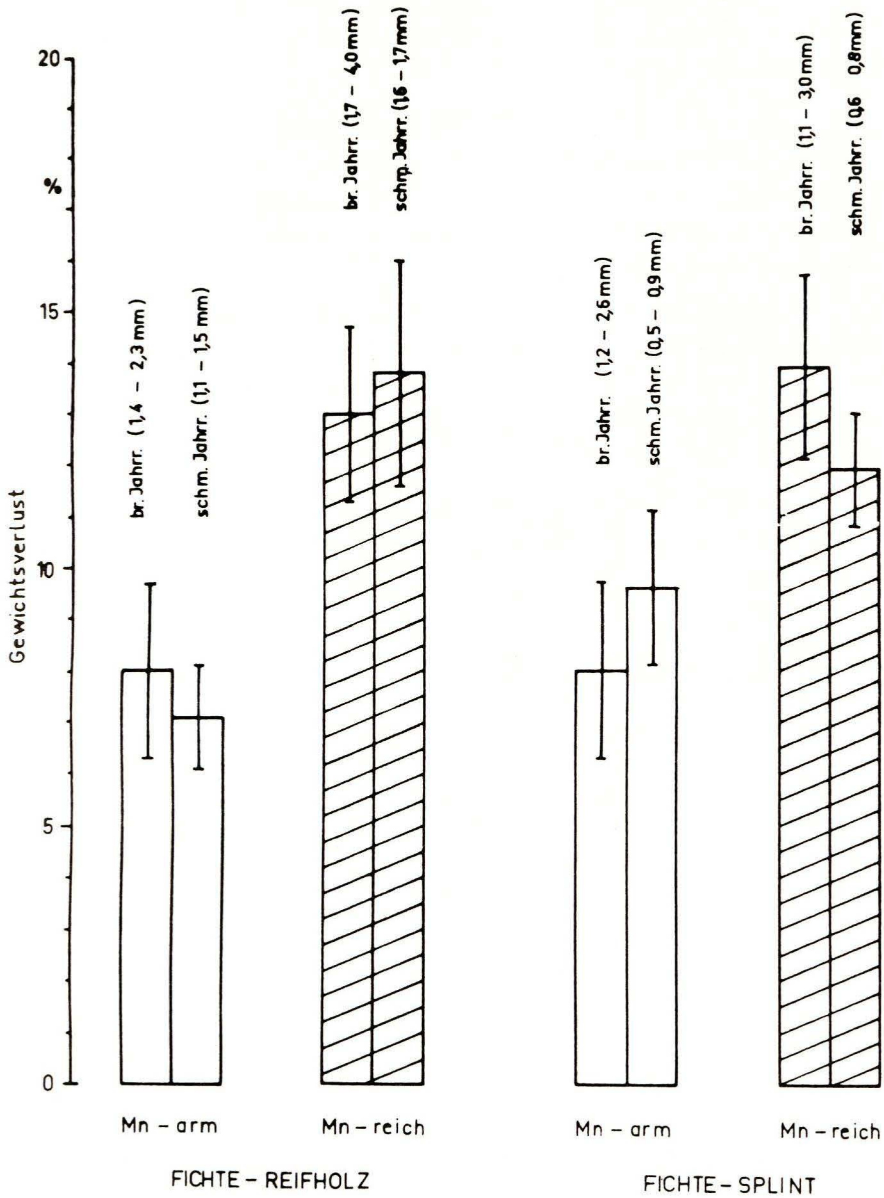


Bild 1.--Vergleich der Gewichtsverluste zwischen Mn-armen und Mn-reichen Fichtenhölzern (<u>Fomes annosus</u> 8, Inkub. 15 Wochen bei 24° C.)

3. In der Abbauleistung des Fomes annosus konnte zwischen Reif- und Splintholz mit breiten Jahrringen im Laborversuch kein wesentlicher Unterschied festgestellt werden.

SUMMARY

1. The influence of manganese was tested on the metabolism of the Fomes annosus. In addition to this we examined sapwood and heartwood from spruce with a different content of manganese. The criticism was the encymatical result of decomposition.

2. The samples from sapwood and heartwood with higher content of manganese showed after incubation also a higher weight loss in relation to the samples with lower content of manganese. The differences are significant. The metabolism of Fomes annosus will obviously favoured by the manganese content of wood.

3. The difference of decomposition between sapwood and heartwood with wide annual rings was in labor test not significant.

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VALUATION OF BUTT ROT OF SPRUCE BY BORING CORES[1/]

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Two hundred and fifty-six 71-year-old spruce trees were analyzed for butt rot by taking cores from the stem at a height of 50 cm. The cores were taken under aseptic conditions and were incubated for 14 days at 20° C. in sterile test tubes under humid conditions, without employing a nutrient medium. For control the spruce trees were cut down afterwards, and the results gained from the cores were compared with the observations on slices taken from the same height. Table 1 gives the total number of trees and the number of healthy and diseased trees detected by the two different test methods.

Employing the bore core method the relation between healthy and diseased trees was 60:40, the true relation 15:85. By the bore core method only 46% of the diseased trees were detected.

Table 2 shows the trees in which the butt rot was caused either by *Fomes annosus* or by other fungi. *Fomes annosus* was the causal fungus in 57% (in 36% alone and in 21% together with other fungi); in 43% other fungal

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1/ These observations will be published in detail and distributed shortly.

species caused the disease. Fomes annosus was detected by the bore core method in 57% of the stems. From 30 stems in which butt rot was not caused by Fomes annosus, three cores yielded Stereum sanguinolentum, three cores S. areolatum and one core Merulius silvester. Out of the remaining 23 cores only Fungi Imperfecti could be isolated or the cores remained sterile.

Table 1

Number of trees	Detected by			
	boring cores		slices	
	number	%	number	%
Total	256	100	256	100
Healthy ones	156	60	40	15
Diseased ones	100	40	216	85

Table 2

Butt-rot causing fungus	Examined by			Sum
	Boring cores	Slices	100+ slices by isolation	
F. annosus alone	70	76	2	78
F. annosus with other fungi	-	40	5	45
F. annosus total	70	116	7	123
Disease caused by other fungus species	30	100+	93	93

Table 3 shows the fungi isolated from the 93 trees with butt rot which was not caused by Fomes annosus (Dr. Siepmann identified these fungi by their cultural characters). From this table it can be seen that the bore core method does not yield satisfying results. Neither can it be employed for the determination of butt rot nor for the identification of causal fungi in old forests.

Table 3

Species of the fungus	Alone	Fungus together with					Results
		C. pu.	*C. sa.*	*M. si.*	*St. ar.*	Impf.	
Coniophora puteana (C. pu.)	-	-	1	-	-	-	1
Coryne sarcoides (C. sa.)	14	-	-	-	-	1	15
Merulius silvester (M. si.)	5	-	-	-	-	-	5
Odontia bicolor (O. bi.)	1	-	-	-	-	-	1
Polyporus stipticus (P. st.)	1	-	-	-	-	-	1
Stereum sanguinolentum (St. sa.)	11	2	1	3	1	2	20
Stereum areolatum (St. ar.)	5	-	-	-	-	-	5
Imperfect Fungi (Impf.)	36	-	-	-	-	-	36

THE INFLUENCE OF LOCAL ENVIRONMENT ON INFECTION BY FOMES ANNOSUS

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and
C. H. Driver
Professor of Forest Pathology, College of Forest Resources, University of Washington, Seattle, Washington

Fomes annosus (Fries) Karst. is the fungus that causes a root- and butt-rot of conifers throughout the temperate regions of the world. Recent surveys show the disease to be widespread and almost exclusively associated with thinned pine plantations in southeastern United States (Driver and Dell, 1961; Powers and Verrall, 1962). In the Pacific Northwest, *F. annosus* is frequently evident in second-growth western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] associated with butt logging scars (Hunt and Krueger, 1962). Recently, this fungus has been found to infect stumps of this host following initial thinning of natural stands (Driver and Wood, 1968). The disease has reached epidemic proportions in sections of the South and is expected to increase in the Northwest as thinnings or other forms of selective cutting become more common in the second-growth stands.

Fomes annosus initially entered stands when spores blown in from distant sporophores landed on, and successfully colonized, freshly exposed stumps or recent basal wounds. The fungus spread into the associated root

system, then into roots of healthy pine trees if they were in close proximity to infected roots. It subsequently spread from one live tree to the others, producing openings of significant size in some plantations or stands.

Where basal wounds, functioning as infection courts for F. annosus, occur during logging in western hemlock stands, their role in the development of infection centers can be reduced by removal of damaged trees and immediate relogging (Shea, 1960). However, the presence of fresh stumps, also infection courts for F. annosus, dictates more complex and intensive procedures to minimize disease development (Driver, 1963).

Of the events between spore infection and tree mortality, the process of initial colonization of stump surfaces was determined to be most readily prevented. The future demise of stand structure through mortality, windthrow, and growth loss could be significantly reduced by deterring stump colonization. The most efficient method was the application of a substance fungicidal to F. annosus on the stumps.

METHODS

The stand description, procedures, and experimental design have been reported (Driver, 1963; Driver and Ginns, 1968; Ross, 1967).

RESULTS

Studies in Britain (Meredith, 1959), Denmark (Yde-Andersen, 1962) and southeastern United States (Driver and Ginns, 1964; Driver and Ginns, 1968; Ross, 1967) showed seasonal fluctuations in the incidence of infection of conifer stumps. Further data have substantiated the existence of an extended period in the 3 years of study when stump infection does not occur (Table 1). It extended for 6 months (May through August) in 1963 and 1965, and prevailed for 9 months in 1964. The date it began or ended varied up to 2 months.

The high ambient air temperatures were suggested (Driver and Ginns, 1964) as influencing colonization of slash pine (Pinus elliottii Englm.) stumps. From 1963 to May 1965, stumps were successfully inoculated with Fomes annosus only when the mean daily temperature was, and had been for approximately 2 weeks, below 70° F. (Table 2). Stumps exposed during

months when mean daily temperature was below 70° F. sometimes became infected; when stumps were exposed and inoculated at the same time rarely all became infected. The number of stumps successfully inoculated varied widely between the different times of cutting, e.g., October 1 versus December 1, 1964.

Table 1.--Percentage of stumps exposed during various months which were naturally infected by Fomes annosus.[1,2]

Year	Month											
	Jan.	F	M	A	M	Jun.	J	A	S	O	N	Dec.
	Percentage											
1963	-	-	-	-	-	0	0	0	0	20	30	10
1964	0	0	0	0	0	0	0	0	0	10	0	20
1965	0	8	0	0	0	0	0	0	4	16	48	80
1966	8	-	-	-	-	-	-	-	-	-	-	-

[1]/Data from February 1965 to January 1966 was taken from Figure 1 of Ross (1967).

[2]/Data from June 1963 to January 1965 based on 10 stumps, that from February 1965 to January 1966 on 25 stumps.

Table 2.--Percentage of stumps yielding Fomes annosus after inoculation with conidia. Each month's data based on 10 stumps.

Year	Month											
	June	J	A	S	O	N	Dec.	J	F	M	A	May
	Percentage											
1963-64	0*[1]/	0*	0*	0*	60	70	100	80	20	0	0	0*
1964-65	0*	0*	0*	0*	90	60	20	0	0	0	0	0*

[1]/Asterisks denote months when Mean Daily Temperatures were above 70° F. prior to time of cutting.

The presence or absence of Fomes annosus was determined by removing wood-chips from randomly selected stumps, culturing them aseptically and recording the microorganisms which grew from the chip. The results were divided into three groups: F. annosus present, F. annosus absent with other fungi present or with no fungi present. Fomes annosus colonization was related inversely to the presence of other fungi. The interrelationships between F. annosus, the local environment and the presence of other fungi was the subject of an earlier paper (Driver and Ginns, 1968).

DISCUSSION

The infection of stumps by Fomes annosus was prevented during the summer months of the tree consecutive years of observations. High ambient air temperatures for extended periods inhibited establishment of F. annosus, no doubt by thermal inactivation of the fungus (Gooding, Hodges, and Ross, 1966). The months when F. annosus was unable to infect stumps varied due to annual climatic fluctuations. In 1964 and 1965 there were nine and six consecutive months, respectively, when infection was prevented. Indications were that the processes regulating stump infection differed in the spring of 1964 from those operating in the same summer-fall period. In the spring, stump analysis (Driver and Ginns, 1968) suggested that fungi that compete with F. annosus, i.e., Trichoderma and Peniophora, were effectively preventing colonization by F. annosus. The thermal inactivation previously mentioned extended further into the fall months than in 1963 and probably 1965.

The maximum number of stumps infected under natural conditions was 40 percent in 1963, 1964 and earlier studies (Driver, 1963). Ross' (1967) report of 48 and 80 percent stump infection in two consecutive months may reflect a marked increase in climatic conditions favoring infection or spore production.

In conclusion, there was found a safe interval of several months when stumps could be exposed with little or no chance of initial infection by F. annosus. This period could effectively be integrated into a disease control program as suggested earlier (Driver, 1963; Ross, 1967). However, practical experiences of the authors on the occurrence and control of F. annosus in pine plantations of the southeastern United Statea and in natural conifer stands of northwestern United States indicated that a thorough analysis of the local environment in relation to the stump infection potential by F. annosus is required before a system of disease control can be made operable for a given conifer species.

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CONTROL AND ERADICATION OF _FOMES ANNOSUS_ IN GREAT BRITAIN

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The importance of _Fomes annosus_ in Great Britain has been indicated by Low and Gladman (1960) and by others. The present paper reports on work carried out in Great Britain since the Second International Conference on _F. annosus_ was held in Scotland in 1960 (Anonymous, 1962). Research has been mainly concentrated on two important aspects of the disease; stump treatment against airborne spore infection and the control and eradication of the disease in crops where it is already present.

PREVENTION OF INFECTION

The Forestry Commission in Great Britain manages 1,500,000 acres of conifer plantations, three-quarters of which has been planted since 1941. The majority of these young plantations have been planted on high moor land ground in the north and west of the country. No tree crops have previously been grown on this land and _F. annosus_ is therefore absent from these sites until thinning operations provide stumps where the fungus can become established. Since 1959, following the work of Rishbeth (1952), it has been the practice to paint the surface of stumps with a protectant to prevent their colonisation by airborne basidiospores of _F. annosus_. The original protectant chosen was creosote, but Rishbeth (1959a) showed and Greig and

and Phillips (In Preparation) have since confirmed that in experiments and in field use creosote was variable in performance and liable to breakdown. Rishbeth (1959b) examined various alternative materials and Punter (1963) carried out further work on the subject. Trials by the Forestry Commission that included some of the more promising of the chemicals tested by Punter showed that a number of substances were superior to creosote, especially when subsequent damage to the stumps occurred, or treatment was incomplete or delayed. As a result of these trials sodium nitrite was selected as the standard stump protectant material to replace creosote in Forestry Commission plantations. Comparisons between sodium nitrite, creosote and control (no treatment) under various conditions can be seen in Figure 1.

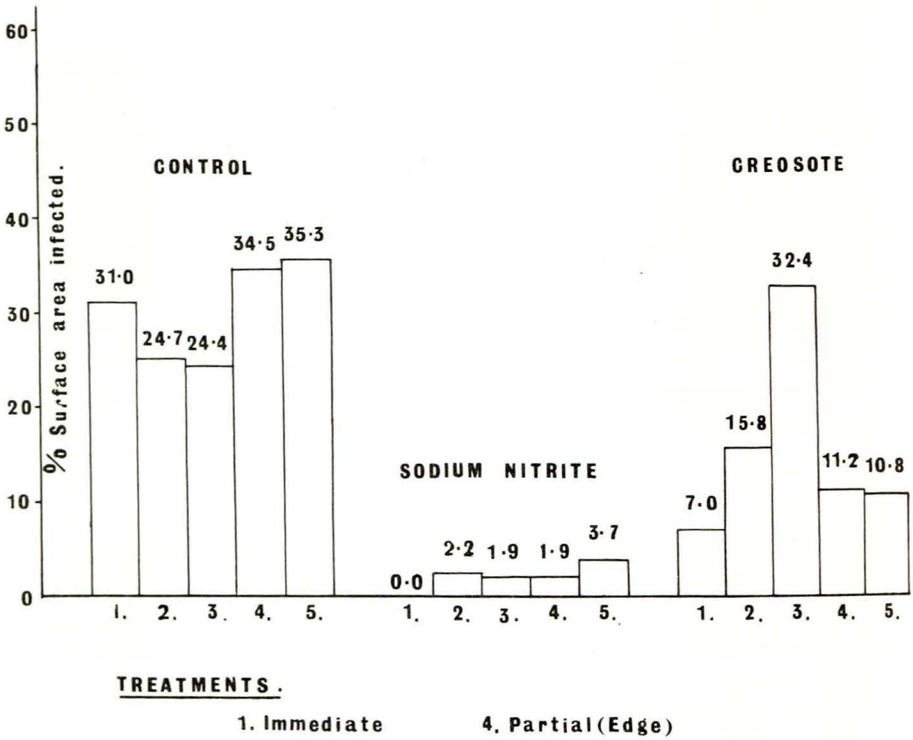

Figure 1.--Control of <u>Fomes annosus</u> on Scots pine by stump protection methods

Sodium nitrite is applied to tree stumps as a 10% solution in water. A marker dye is added to the solution to stain the wood, which enables a check to be made that complete coverage of the stump has been achieved. Sodium nitrite is a toxic substance and precautions are required when handling and storing the chemical. Sodium nitrite is now used throughout the Forestry Commission's plantations with the exception of certain water catchment areas and in plantations from which it is impossible to exclude livestock. For these areas alternative materials are required and an experiment was designed to distinguish between a number of substances, all of which had given good protection against F. annosus in previous trials. Seven protectants were tested in this trial: disodium octoborate (20%); urea (20%); ammonium sulphamate (20%); sodium nitrite (10%); Peniophora gigantea inoculation; and the herbicides Reglone (containing diquat), and Gramoxone (containing paraquat) both at 5.5%. Four separate trials were laid down to test for possible failures of protection. Results of these experiments are being prepared for publication (Greig and Phillips, In Preparation) and showed that all the protectants gave very significantly better results than control (no treatment). No significant differences could consistently be found among the treatments. The results are summarised in Figure 2.

These results suggest that the various treatments are equally effective in preventing F. annosus basidiospores from infecting stump surfaces. The choice of protectant for first rotation unthinned plantations could be made from any of the above materials taking account of such factors as cost, toxicity, ease of handling, etc.

For plantations where F. annosus is already established, other factors must be taken into account when selecting a stump protectant material. Protectants such as disodium octoborate allow a build-up of F. annosus in roots of stumps, due to the absence of competing fungi which are excluded from the stump surface. For pine areas, Peniophora gigantea inoculation is recommended (Rishbeth, 1963). Until further experiments have been laid down to determine the effect of the protectants on established infection by F. annosus, the use of sodium nitrite as the standard protectant for use in the Forestry Commission's plantations will continue.

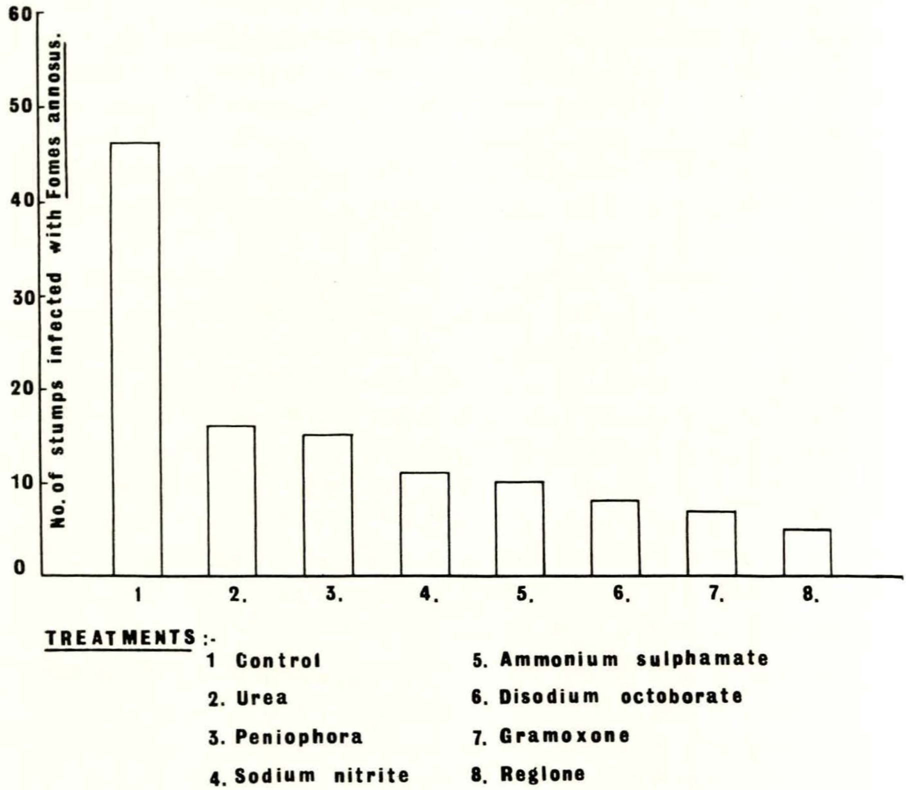

Figure 2.--Summary of results from stump protectant trials

CONTROL AND ERADICATION OF F. ANNOSUS INFECTION BEFORE REPLANTING INFECTED SITES

As a result of Peace's paper (Anonymous, 1954) presented to the First Conference on Root and Butt Rots caused by Fomes annosus at Wageningen in 1954, a number of long-term experiments on the eradication of F. annosus in infected sites before replanting were laid down in Great Britain. There are three experiments in the first series as follows:

1. 30-year-old European larch, Lael Forest, Scotland.
2. 30-year-old Sitka spruce, Kerry Forest, Wales.
3. 24-year-old Scots pine, Harling Forest, England.

The experiments are all long-term and those at Lael and Kerry are primarily concerned with the susceptibility of various tree species to butt rot.

Assessments of the levels of rot will be made on all trees felled for thinnings, but even early results will not be available for at least another 10 years.

The Harling experiment is on an alkaline site and the following treatments were included: (a) Control (no treatment); (b) Girdling of trees six months prior to felling; (c) Poisoning of trees prior to felling using sodium arsenite; (d) Poisoning the stumps with sodium arsenite; and (e) Stump removal. An annual assessment is made of the mortality of the replanted Scots pine trees, recording the number of trees killed by F. annosus and a cumulative total is kept for each plot. The first assessment was made when the plants were four years old. Figure 3 shows the increase in deaths from year 4 to year 11 in the untreated control plots and in those plots from which stumps were removed. The remaining treatments did not differ significantly from the control [treatment (a)] and therefore were omitted. The stump removal treatment reduced the level of killing by F. annosus to 20% from 54% in the control. This reduction is very significant.

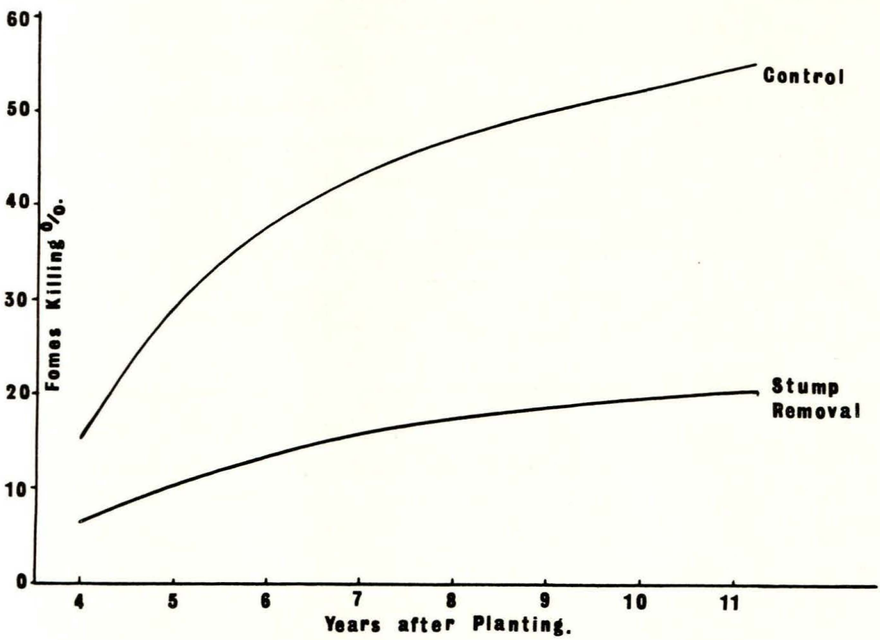

Figure 3.--The effect of stump removal on the level of killing by F. annosus

The Harling experiment was also designed to test the effect of delayed replanting on the level of killing. Plots have been replanted at 0, 2, 4, 6, 8, 10 and 12 years after felling. Figure 4 shows the mortality rate in the 0-, 2-, 4-, 6-year delay plots when the trees were all 6 years old. Also shown in Figure 4 are the average tree heights for the plots. At 6 years the heights for the 0-, 4- and 6-year delay plots are all comparable at 4.61, 4.37 and 4.15 feet, but the height of the 2-year delay plots are much lower at 2.39 feet. The level of killing by F. annosus is also very much lower at 11.6%. It is assumed that root production and spread is related to height growth and therefore the low level of killing in the 2-year delay plots is related to reduced root contacts with infected stumps and not to a reduction in F. annosus losses due to the delayed replanting. The reduction in losses for the 4- and 6-year delay plots can therefore be related to the delayed replanting because the heights of the trees in these plots are comparable to the 0-year delay plots at the same age.

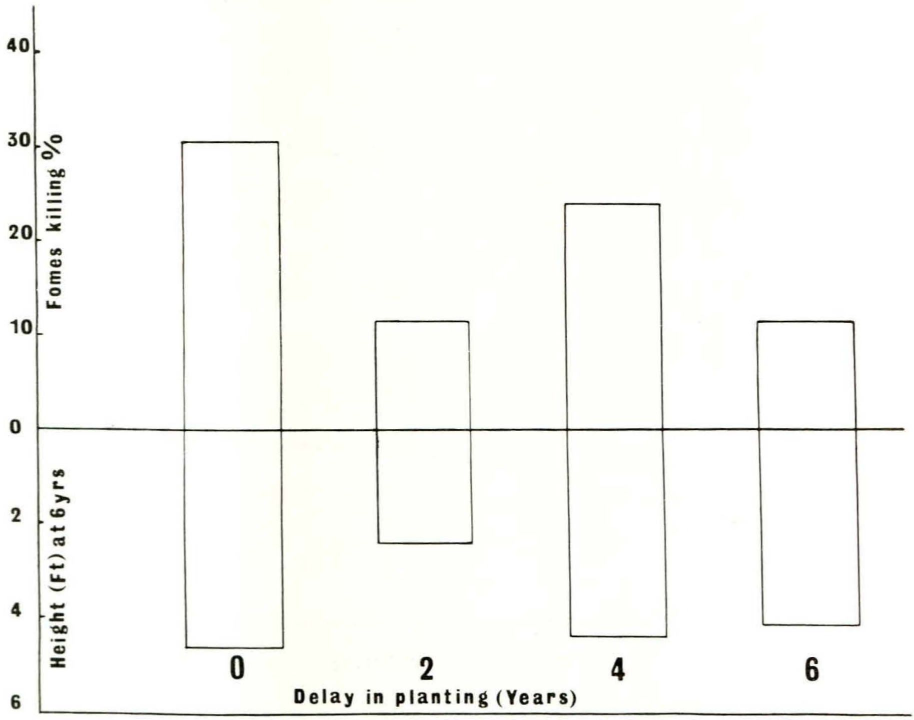

Figure 4.--The effect of delayed replanting on the level of killing by F. annosus

The Harling experiment discussed above was laid down before Rishbeth (1963) developed the technique of inoculating pine stumps with P. gigantea. In later experiments, again on Scots pine, P. gigantea has been tested along with a number of chemical stump protectants. Table 1 below shows the level of killing by F. annosus in the replanted Corsican pine for two experiments 7 years after planting.

Table 1

Treatment	Experiment A	Experiment B
Control	13.8	16.0
Stump removal	2.6	2.5
Peniophora gigantea	12.6	8.0
Ammonium sulphamate	13.7	8.9
Urea	17.6	13.6
Disodium octoborate	14.8	10.8
Creosote	17.5	12.1

The stump removal treatment is the only one to drastically reduce the number of trees killed by F. annosus. There is a suggestion in the figures that P. gigantea and ammonium sulphamate [which favours colonisation by P. gigantea (Rishbeth, 1959b)] may also reduce the killing by F. annosus.

Inoculation of pine stumps with P. gigantea in a badly infected stand can reduce the very heavy build-up of infection in the stumps which would otherwise occur; Figure 5 illustrates this point. In the absence of P. gigantea, F. annosus occupied 80% of the roots of Scots pine, but with P. gigantea inoculation the F. annosus infection was reduced to 46%.

All the long-term experiments to control F. annosus in second rotation crops are still in their infancy and we can only tentatively put forward possible lines of action. Stump removal will almost certainly reduce the amount of killing by F. annosus to acceptable levels. The cost of mechanically digging out stumps in situ is estimated to be in the region of 20 to 30 pounds per acre. An alternative and possibly cheaper method may be to winch over whole trees and then to remove the stump and roots. Comparative cost trials of various methods of stump removal are planned for the autumn of 1968.

The two alternative treatments to stump removal are stump treatment with P. gigantea and delayed replanting.

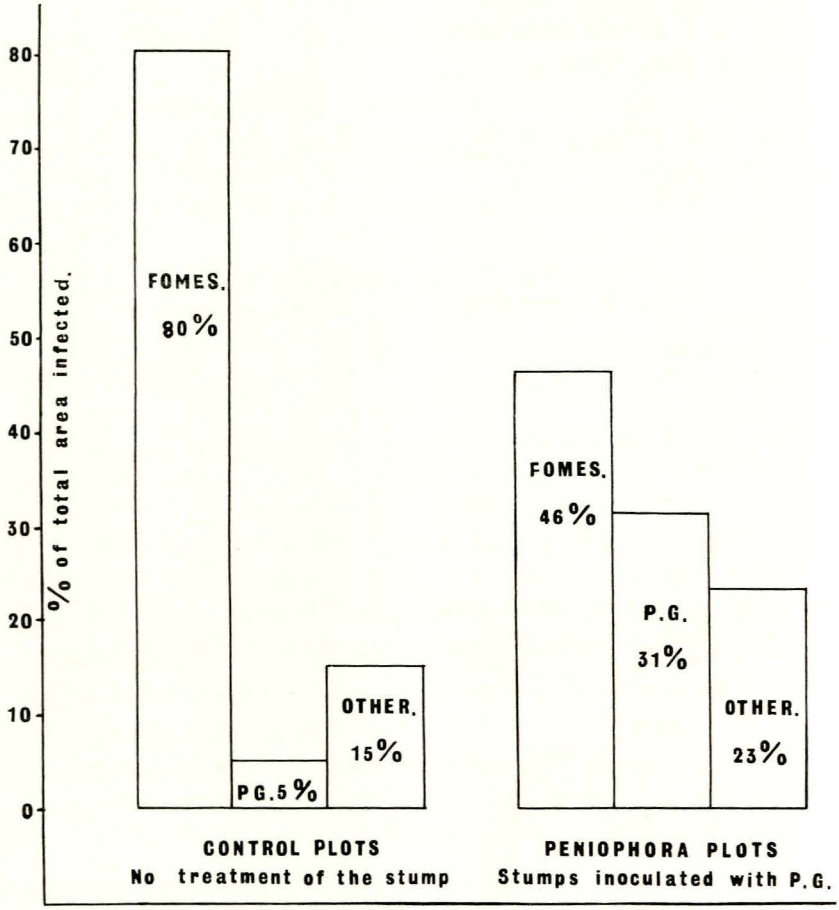

Figure 5.--Distribution of Fomes annosus and Peniophora gigantea in the roots of Scots pine stumps 3 1/2 years after felling

A modification of the routine stump treatment procedure with P. gigantea is being included in an experiment at present being laid down. This involves the inoculation of P. gigantea into stump tissues through vertical chain saw cuts into the stump and may result in a more rapid and extensive colonisation of the stumps by P. gigantea.

Delayed replanting in combination with P. gigantea stump treatment may give an acceptable stocking in the second rotation crop.

The comparative costs of these treatments and their potential benefits are vitally important before a decision can be made to adopt a particular

treatment. A preliminary attempt has been made to study the economics of two eradicative treatments and the result is attached as an appendix.

SUMMARY

Work on Fomes annosus by the Forestry Commission Research Branch has been concentrated on stump treatments against airborne infection and the control and eradication of the disease in crops where it is already present.

Sodium nitrite is now used as the standard stump protectant in British forests (except for pine plantations), but because of its toxic nature other chemicals are used in special circumstances. Further information is required on the use of chemical stump treatments in areas already infected by F. annosus.

Peniophora gigantea is recommended for stump treatment in pine plantations.

Three methods have been found experimentally for eradicating F. annosus from previously infected pine stands; stump removal, delayed replanting and stump treatment with P. gigantea. These methods have worked with varying degrees of success and the ultimate choice of the field control method will now largely depend on a detailed economic study of the alternative methods available.

APPENDIX

Economic Aspects of the Eradication of Fomes annosus

There is sufficient experimental evidence on the effects of two remedial treatments--stump removal and delayed replanting--to make a preliminary economic assessment of costs and benefits.

Studies of net returns from British forest plantations are based on calculations of N. D. R. (Net Discounted Revenue). The forecast value of a crop at clear felling (taking thinnings into account) is discounted back at a specified rate of interest to the year of planting to give the Discounted Revenue. The costs of planting and establishment are then subtracted from this to give the net discounted revenue. Apart from special treatments, the routine costs of planting remain constant in the considerations which follow and we shall therefore be mainly concerned with discounted revenue.

Losses caused by F. annosus may reduce the discounted revenue and thus the costs of a remedial treatment can be offset against the additional revenue that will be produced by this treatment.

Table A shows the additional revenues which can be expected at two rates of discount for the experimental treatments assuming losses of 50% (40-60%) with no treatment, 35% (25-45%) with delayed replanting and 20% (15-30%) with stump removal.

Thus at the normal 3 1/2% discount rate, the additional revenue achieved by delaying planting for four years would be 15 pounds per acre (ranging from 0 to 45 pounds). The figure for discounted revenue (130 pounds) takes into account the reduced revenue caused by the four year delay and the additional expenditure of 5 pounds per acre is put down for additional weed control associated with delayed replanting. Therefore at the 3 1/2% discount rate an extra 10 pounds per acre is made on average if one delays replanting for four years.

Similarly with the stump removal treatment, assuming an expenditure of 20 pounds per acre on the treatment and a discount rate of 3 1/2 per cent, there is an average benefit of 15 pounds per acre.

Table A shows the range of figures which can be achieved with varying losses and at two rates of interest.

The calculations made to produce the figures in Table A involve several other assumptions which include:

1. The losses are grouped and not scattered (the calculations were based on loss distributions in the experiment).

2. The quality and therefore value of the timber will vary depending on the distribution of trees remaining after losses have occurred.

3. The spacing of the replacement crop is 6 ft. x 7 ft.

We are most grateful to Mr. P. A. Wardle (Forest Economist) and Mr. G. Hamilton (Mensuration Officer) for undertaking these calculations and helping to interpret the results.

Table A.--Costs and benefits for eradication of Fomes annosus - pounds per acre

Treatment	Discount Rate: 3 1/2% Additional Expenditure	3 1/2% Discounted Revenue	3 1/2% Additional Revenue	5% Additional Expenditure	5% Discounted Revenue	5% Additional Revenue
No treatment	0	115 (100-130)	0	0	55 (45-65)	0
Delayed re-planting for four years	5	130 (115-145)	15 (0-45)	5	65 (55-75)	10 (0-30)
Stump removal	20	150 (140-155)	35 (10-55)	20	75 (70-78)	20 (5-35)

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THE ACTUAL SITUATION OF RESEARCH AND CONTROL OF THE ROOT ROT FUNGUS (FOMES ANNOSUS) IN THE NETHERLANDS

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Since the second conference on *Fomes annosus* held under the auspices of section 24 (Forest Protection) of the International Union of Forest Research Organizations in North- and East-Scotland from May 30-June 4, 1960, research on *Fomes annosus* in the Netherlands made a considerable progress.

Some aspects of research executed and progress made concerning control of the root rot fungus are mentioned below.

The first step to be done in regard to the control of *Fomes annosus* was to obtain more detailed knowledge on the incidence of this fungus in our country. Therefore a circular letter entitled "root diseases in coniferous trees" has been forwarded to all State forest district officers and many private forest owners towards the end of 1960. In this circular letter we asked for detailed information on the type of root rot occurring in coniferous stands in the forest areas under their administration. In view of the desirability to have these centres of infection carefully localised we insisted on the use of managements maps and the use of the following symbols in the forest areas where root rot has been observed: Z = for general use when type of root rot is unknown or doubtful; FA = root rot

caused by Fomes annosus; AM = root rot as a result of Armillaria mellea; RU = root rot as a result of Rhizina undulata, and W = windblow as the result of root rot of various causes.

On this inquiry a considerable number of maps has been received. However, as we knew that much confusion exists in regard to a positive recognition of Fomes annosus, the next step to be done comprised a visit to nearly all localities mentioned in order to check the reports. Towards the end of 1963 this inventory work was finished so that we could visualize the actual situation of damage by Fomes annosus in coniferous stands.

As had already been assumed the term "root rot" has a general significance in practice, since it may imply various causes, but nevertheless it appeared that Fomes annosus is one of the fungi very often connected with root rot, although in many cases it was confused by attacks brought about by the fungi Armillaria mellea or Rhizina. In some cases even damage had been observed in older trees caused by Polyporus schweinitzii.

The general picture of root rot damage in the Netherlands can be characterized as follows:

Root attack by Fomes is observed in various species of coniferous trees. It may occur as root rot, butt rot or windblown trees.

In Scots pine dieback by Fomes may already occur in young trees; in older stands it is well-known and very common by formation of group-dying. Windblow as a result of root rot by Fomes has been observed in some 50-year-old Scots pine trees on sites consisting of loess or loamy sandy soils.

In Japanese larch Fomes may be the cause of butt rot, especially in trees of 20 years and older. This type of rot may considerably increase when stands of Japanese larch are established on old agricultural sites.

Douglas fir may develop a serious dieback in young trees, especially when planted after a Scots pine rotation. Older stands may suffer to windblow as a result of root rot by Fomes, particularly on old agricultural sites.

Norway spruce may only incidentally be killed by Fomes, but as a rule develop a serious extending butt rot.

Now and then attack by Fomes has been observed on following coniferous trees: Picea sitchensis, Thuja plicata, Tsuga species and Juniperus species. On hardwood trees it may occur on American oak, alder and Prunus serotina.

To make a beginning in the control of the root rot fungus, practice had to be convinced of its necessity and the possibilities of control by treating stumps. At this time research and experience with chemical substances such as polyborchlorate were in the very beginning, whereas creosote had already proved its effectiveness against stump infection by basidiospores of Fomes. This has been the reason to advise stump treatment by means of creosote in thinnings as well as in clear-cut fellings in pine, larch, Douglas fir and Norway spruce. However, as soon as a serious root rot can be observed in existing stands the application of creosote in thinning procedures and fellings has been dissuaded, instead of this leaving the stumps unprotected. Extraction of stumps has been advised after a clear-cut felling in heavily infected areas.

The application of stump treatment in practice, however, is still in its very beginning. In some forest areas use of creosote is common practice, especially there where damage by Fomes could directly be demonstrated, in other cases the interest for stump treatment is often very poor.

So far we did not consider the use of sodium nitrite as a stump treatment, although this means is in common use in Great Britain. Up till now we preferred creosote since this gave us the possibility of checking the stumps treated. Recently, however, Thompson and Capper manufactured sodium nitrite to which blue stain has been added and we now hope that this substance can replace creosote. It has one disadvantage being very poisonous and therefore we are afraid that it cannot be used in those forest areas in the Netherlands where deer are protected.

In the laboratory research on Fomes has dealt with several aspects in the biology and control of this organism. One special aspect is the control by means of the competitive fungus Peniophora gigantea. We investigated its possibility to colonize severed, fresh roots of pines, the rapidity of mycelium growth in culture, the interaction between Peniophora and Fomes in vitro and methods of large scale propagation of the fungus Peniophora on various media.

In addition to the laboratory work some field experiments have been laid down with Peniophora using oidial suspensions for spraying stumps after a clear-cut felling in comparison with stump treatment by creosote. In one project recently started the development of Peniophora gigantea on Scots pine stems is investigated by topping trees instead of thinning in a normal way and spraying remaining stems with oidia. This has been done in a forest area where the air-spora contents of Fomes are very high, making a normal thinning without stump treatment very risky. By cultivation of Peniophora on the dying stems we hope to raise the quantity of spores of Peniophora in this area and to minimize infection of stumps by Fomes in later periods.

The following papers dealing with Fomes annosus and Peniophora gigantea have been issued in the Netherlands since 1960 by J. Gremmen:

Biologie en bestrijding van de wortelzwam, Fomes annosus (Fr.) Cooke. (with an English summary). Ned. Bosb. Tijdschr. 32: 394-409, 1960. Korte Meded. Bosbouwproefstation, Wageningen, Nr. 44, 1960.

De huidige stand van het wortelzwamvraagstuk in Nederland. T. Ned. Heidemaatschappij 73: 306-312, 1962.

De biologische bestrijding van de wortelzwam, Fomes annosus (Fr.) Cooke door middel van Peniophora gigantea (Fr.) Massee. (with an English summary). Ned. Bosb. Tijdschr. 35: 356-367, 1963. Korte Meded. Bosbouwproefstation, Nr. 59, 1963.

COLOR INFRARED PHOTOGRAPHY EFFECTIVELY DETECTS PINES KILLED BY FOMES ANNOSUS

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ABSTRACT

Ektrachrome Infrared Aero Film, type 8443, provides a good basis for detecting pines killed by _Fomes annosus_ root rot. It did not distinguish living, infected pines and healthy pines.

Past methods for detecting and counting pines killed by the root and butt rotting fungus, _F. annosus_ (Fr.) Cke., involved ground inspections, sketch mapping from low-flying aircraft and mapping of infection centers from helicopters. These methods are costly and (with the exception of ground inspections) often inaccurate.

More recently aerial photography has gained recognition as a relatively inexpensive, rapid, and accurate survey method. The photographs have the added advantage of being suitable for other forestry uses, such as measuring timber volume and identifying soils and drainage systems.

This study was performed to determine the value of Kodak Infrared Aero Film, type 8443 (Ektrachrome IR), as an aid in detecting and counting dead and dying trees infected by _F. annosus_.

FILM CHARACTERISTICS

Ektrachrome IR film is a much improved version of Aero Kodacolor Reversal Film, Camouflage Detection. It is much more stable and faster (Anonymous, 1964; Fritz, 1967). Unlike black and white or normal color films, Ektrachrome IR is sensitive to radiation in the near infrared region of the electromagnetic spectrum. Black and white and normal color films have an electromagnetic spectral sensitivity range of 400-700 mμ, similar to visible light. Ektrachrome IR's range of 500-900 mμ records visible green and red, plus infrared in the 700-900 mμ range.

This film's 3-dye layers are sensitive to green, red and infrared radiation, which is recorded as blue, green, and red, respectively. Thus healthy foliage, which reflects infrared energy, appears red on the transparencies. Dead pine trees with yellow or red foliage appear yellow or green in the infrared photographs. Dead trees which have shed all foliage appear blue.

It is important to note that all 3 layers are sensitive to blue radiation, so a yellow filter must be used on the camera to exclude this blue light.

CHARACTERISTICS AND MECHANICS OF FOLIAGE REFLECTANCE

Figure 1 illustrates the fact that most reflected energy from healthy foliage is infrared. Infrared radiation is reflected in tree foliage at the cell wall-air cavity interfaces within the loosely arranged spongy mesophyll (Knipling, 1967). When a plant is subjected to a severe stress, such as that caused by disease, the spongy mesophyll cells evidently collapse or become plugged with fungal or host reaction products, eliminating many of the cell wall-air cavity interfaces.

At this point, infrared reflectance drops off and the dead or dying foliage is recorded as green on the transparency. In some plants, particularly broad-leafed species, this decreases infrared radiation reflectivity before any color change is apparent to the human eye. In these cases, Ektrachrome Infrared provides the earliest possible warning of disease development.

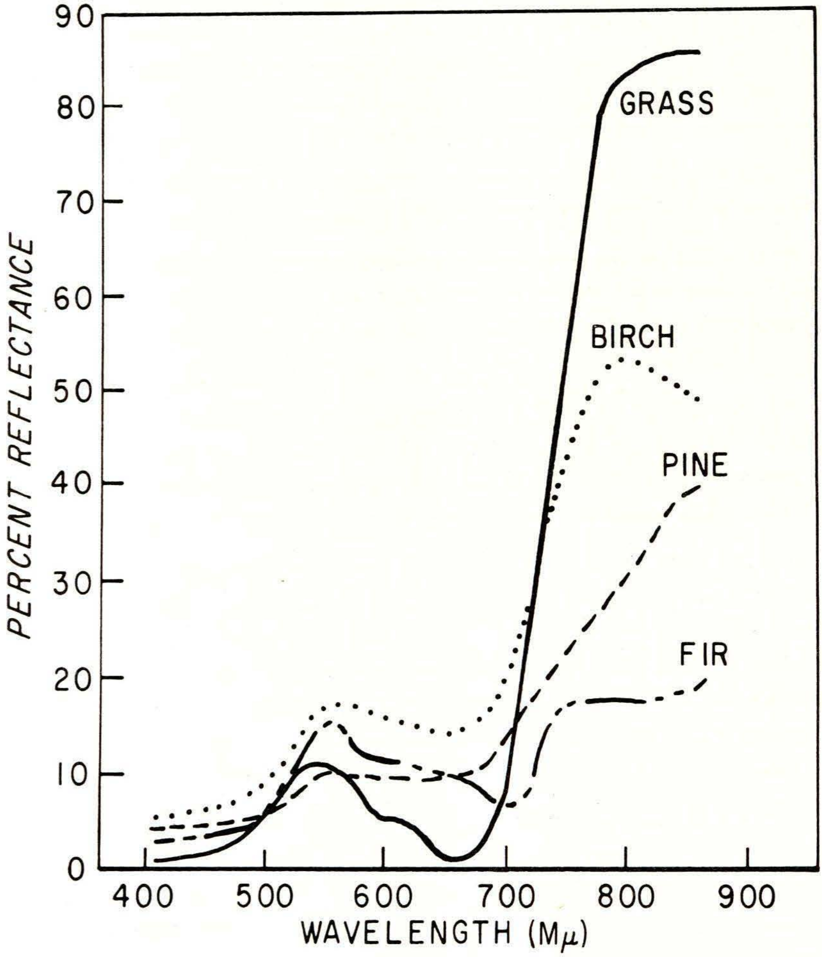

Figure 1.--Spectral reflectance curves of various typical foliage types. From Fritz (1967).

PROCEDURES

The studies were conducted in southern Illinois, Rhode Island, and eastern Connecticut by the Delaware, Ohio, and Amherst, Massachusetts, Field Offices of the Division of Forest Pest Control, Forest Service, U. S. Department of Agriculture. The photography was done on 5 infected shortleaf

pine (Pinus echinata) Mill. plantations in Illinois and 11 infected red pine (Pinus resinosa) Ait. plantations in Rhode Island and eastern Connecticut.

Photography was scheduled in August and September 1967--dead pines retain yellow or red foliage at this time of year, and deciduous trees are still green. This eliminates the possibility of confusing dead pines with bare hardwoods. The Illinois photographs were taken at a scale of 1:3200; those in Rhode Island and Connecticut were taken at a scale of 1:1600.

Suspect trees, which appeared yellow, green, or blue were encircled on the photos with wax pencil, then visually checked in the plantations.

Ninety-nine yellow and green trees were marked on photographs of the Illinois plantations; 106 red-topped trees were found on the ground. In this case 93.4 percent of the dead trees were located by aerial photographs (Hanson and O'Brien, 1968).

The photographic system proved 100 percent effective in the Rhode Island and Connecticut plantations. The 150 trees marked on the transparencies were found to be dead when observed on the ground, and no other dead trees were recorded (Hadfield, 1968).

In a separate study in Rhode Island and Connecticut red pine plantations, we tested Ektrachrome IR's ability to detect annosus root rot in trees retaining green foliage. Fifty living infected trees were first located on the ground. These had sparse, stunted foliage and were adjacent to sporophore-bearing pines. The infected trees were then located on the photos, and their photographic color was compared with that of healthy and dead trees.

This comparison indicated that infected, but still living, trees do not show as such on the photographs. Regardless of the health of a living red pine, if it had green foliage, its image always appeared dark red on the photographs. This may be attributable to the compact nature of red pine needles, which is unlike hardwood foliage in this respect. Collapse of cell wall-air cavity interfaces apparently occurs much more readily in hardwoods, reducing infrared reflectivity. Since this doesn't occur in the red pines, infected individuals continue to photograph red as long as they have green foliage.

DISCUSSION

The results indicate that Ektrachrome Infrared Film is a useful aid in detecting and counting dead pines infected with F. annosus. Not all red-topped trees were observed on the Illinois photos because some were overtopped by other trees. Wide spacing of trees eliminated this type of problem in the Rhode Island and Connecticut plantations.

Causal agents of damage cannot be identified from aerial photographs. We are presently developing a sampling system that will incorporate photo-interpretation data and a minimum of ground analysis. This should provide an accurate survey system that should be of great value for surveying large areas.

Ektrachrome Infrared is more useful for detecting and counting dead pines than other available films for two reasons:

1. The color contrast on the infrared photographs is highly dramatic. Arrays of reds, yellows, greens and blues that appear on the photos are foreign to the human eye and, thus, small differences in colors and shades are striking and easily picked out.

2. Infrared film can be exposed on hazy days with no detrimental effect. The yellow filter removes the haze, which consists of scattered blue light. This cannot be done with regular color film or black and white film. The film's haze-penetrating ability enables photographers to use it at high altitudes, thereby increasing photographic coverage.

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EVALUATION OF STUMP TREATMENT CHEMICALS FOR CONTROL OF FOMES ANNOSUS

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In 1962, plots were established at 8 locations throughout the southeastern United States to evaluate the effectiveness of ammonium fluoride and creosote in preventing stump infection by Fomes annosus (Fr.) Karst. Ammonium fluoride was chosen as a test chemical because it stimulates the development of Trichoderma spp. (Verrall, 1949) which are known antagonists of many pathogens. Creosote was selected on the basis of its reported success in England (Rishbeth, 1959) in preventing stump infection by air-borne basidiospores of F. annosus. Creosote (American Wood Preserver's Assoc. No. 1 distillate) and ammonium fluoride solution (4% w/v) were applied by hand sprayer to the point of runoff immediately following cutting. Stumps in some plots were inoculated with a conidial suspension of F. annosus following treatment.

Treatments were evaluated 3 months later by culturing chips taken from the top 2 to 3 inches of the stump on a medium selective for F. annosus (Kuhlman and Hendrix, 1962). Only one plot had sufficient stumps infected with F. annosus at the end of 3 months to evaluate the chemicals. At this time, F. annosus was present in 95, 8, and 4 percent of the check,

creosote-, and ammonium fluoride-treated stumps, respectively. The top 2 to 3 inches of the creosote-treated stumps were essentially sterile. Trichoderma spp. had colonized almost completely the upper portions of the fluoride-treated stumps and the cut surface of many stumps was completely green with spores of this fungus.

The plot just described was established in October; all other plots were established in late spring or early summer. Temperatures during this latter period often reach levels which may prevent stump infection by F. annosus (Gooding, Hodges, and Ross, 1966), and this factor probably was responsible for lack of infection in most of the plots.

In 1965, approximately 3 years after treatment, stumps and roots were again checked in the plot established in October. At this time, F. annosus was found in roots from 63, 83, and 97 percent of the check, ammonium fluoride-, and creosote-treated stumps, respectively. About 20 percent of the infected stumps had sporophores of the fungus.

The reason for the apparent breakdown in stump protection was not known, but several possibilities existed: (1) the level of infection in the treated stumps was so low after 3 months that the sampling procedure used failed to indicate infection; (2) infection took place after 3 months in the treated stumps; (3) the treatments were effective in preventing stump surface infection but infection occurred in some other manner; (4) colonization of the fluoride-treated stumps by Trichoderma masked any F. annosus present.

Evaluating the effectiveness of stump treatment chemicals has been done in a similar manner by most workers. It usually involves removing the top 2 to 3 inches of the stumps 3 to 6 months after treatment and either incubating the entire stump top in a moist chamber (Morris and Knox, 1962; Rishbeth, 1959; Sinclair, 1964), or splitting it in several pieces, removing small chips, and plating them on various culture media (Berry, 1965; Driver, 1963). For the most part, reports on effectiveness of stump treatments have been made on the basis of the first sampling. No information is available on colonization of stumps and their root systems by F. annosus and other microorganisms for long periods after treatment. For this reason, the following study was initiated to re-evaluate the reliability of the common method of determining the effectiveness of stump treatment chemicals over a long period of time, as well as to determine the reason for failure of ammonium fluoride to prevent stump infection.

MATERIALS AND METHODS

Two chemicals were used in the study. Ammonium fluoride was again used as a chemical which encourages growth of certain fungi. Borax was chosen because it, like creosote, prevents stump colonization by most organisms and has been shown, on the basis of short-term studies, effective against F. annosus (Driver, 1963). Creosote was not used again because of the safety hazards involved in its application.

Plots were established in a 20-year-old plantation of Pinus elliottii Engelm. var. elliottii located in the Sandhills area of South Carolina. The plantation was thinned in February 1965 to about 50 percent of its original basal area. Technical grade borax was applied dry in the granulated form in quantities to lightly cover the stump surface. A 4-percent solution (w/v) of ammonium fluoride was applied to the point of runoff with a sprayer. Treatments were applied immediately following thinning. A few minutes later, all stumps were inoculated with a conidial suspension of F. annosus. Non-treated, inoculated stumps served as checks. Individual plots consisted of 10 stumps in a row for each treatment. Three replicates of each treatment for each sampling period were distributed randomly throughout the plantation, making a total of 30 stumps sampled for each treatment at each sampling period.

To determine the relative amount of natural inoculum present, traps of freshly cut pine stem sections approximately 100 mm. in diameter were exposed for 8 hours during the time the plots were established. An average of five colonies of F. annosus per trap developed after 7 days, indicating a rather low level of inoculum availability.

Stumps were sampled 1, 3, 6, 9, 12, and 18 months after treatment. At each sampling period the stump was severed at a point just above the uppermost lateral roots, which was 4 to 12 inches below the stump surface. Two lateral roots, regardless of size, were randomly selected from each stump, severed, and removed from the soil. Stumps and roots were then taken to the laboratory for culturing.

In the laboratory, the stumps were immersed in 0.5 percent sodium hypochlorite and carefully split into quarters with an axe. Cores were removed radially at 1-inch intervals across the tangential faces of two of the quarter sections with a Swedish increment hammer. For the first two sampling periods, cores were taken vertically at 1-inch intervals down the tangential faces; at 6 and 9 months, cores were taken at 2-inch intervals.

No samples were taken from the stumps after 9 months because F. annosus had almost disappeared from the stumps at that time.

Roots were cut into 18-inch sections, immersed in 0.5 percent sodium hypochlorite, and carefully split in half. Two cores were removed at 3-inch intervals for the length of the root for roots larger than 1/2-inch diameter; only one core was removed from smaller roots.

All cores were plated on a medium especially formulated for isolating F. annosus from wood (Kuhlman and Hendrix, 1962). Observations were made after 7 to 9 days.

RESULTS

After 1 month, F. annosus was recovered from 90 percent of the check stumps, 40 percent of the fluoride-treated stumps, and only 3 percent of the borax-treated stumps (Table 1). The fungus was present only in the upper 1 inch of the stumps at this time. Other fungi were only occasionally recovered from the stumps at this time. No microorganisms were isolated from the roots.

After 3 months, F. annosus was isolated from 86, 69, and 0 percent of the check, fluoride-, and borax-treated stumps, respectively. In the check stumps, F. annosus was isolated down to 5 inches from the stump surface, but only to 4 inches in the fluoride-treated stumps. Peniophora gigantea was isolated from 90 percent of the check stumps, but was present for the most part only in the upper 2 inches. This fungus was not isolated from treated stumps. Trichoderma spp. were isolated from only a small percentage of the check and borax-treated stumps but were present in 73 percent of the fluoride-treated stumps. The upper surface of many of the fluoride-treated stumps was green from heavy Trichoderma sporulation. Although F. annosus was present in 69 percent of the fluoride-treated stumps, it was isolated primarily at the 4-inch level, below the area colonized by Trichoderma.

F. annosus was isolated from two roots of check stumps and two roots from fluoride-treated stumps after 3 months. The fungus was present in a portion of the root 0-3 inches from the root collar in three roots and 0-18 inches from the root collar in the remaining root. Although it is possible that infection in three of these roots came from the main stump body, the presence of the fungus 18 inches from the root collar at this time is

Table 1.--Percentage recovery of *Fomes annosus*, *Peniophora gigantea*, and *Trichoderma* spp. from stumps and roots of stumps treated with ammonium fluoride and borax and from non-treated checks

Months after treatment	Borax						Ammonium fluoride						Check					
	Stumps			Roots			Stumps			Roots			Stumps			Roots		
	F	P	T	F	P	T	F	P	T	F	P	T	F	P	T	F	P	T
1	3	0	A[1/]	0	0	0	40	0	A	0	0	0	90	0	A	0	0	0
3	0	0	3	0	0	0	69	0	73	10	0	0	86	90	23	6	0	0
6	0	0	23	0	0	18	13	10	83	15	18	13	27	73	33	60	6	15
9	0	3	30	0	3	13	0	17	83	23	23	17	7	47	47	40	23	6
12	-	-	-	10	10	10	-	-	-	43	43	23	-	-	-	23	77	23
18	-	-	-	0	7	24	-	-	-	39	14	26	-	-	-	18	46	33

[1/] A = Isolated occasionally
F = *Fomes annosus*
P = *Peniophora gigantea*
T = *Trichoderma* spp.

evidence of direct root infection in the fourth root. With the exception of a few bacteria, no other organisms were isolated from the roots at the 3-month sampling period.

The number of stumps from which F. annosus was isolated had declined considerably 6 months following treatment, being present in 27, 13, and 0 percent of check, fluoride, and borax treatments, respectively. When present, it was usually found at the lower levels within the stumps. The upper levels of the check stumps were colonized primarily by P. gigantea with some Trichoderma spp. The reverse was true for the fluoride-treated stumps, where Trichoderma spp. were dominant and P. gigantea was isolated only occasionally. As was true after 3 months, F. annosus was usually recovered below the area colonized by Trichoderma. Trichoderma spp., Fusarium moniliforme Sheldon, and a few bacteria were occasionally isolated from the borax-treated stumps. Much of the area of stumps treated with borax was still free of microorganisms at this time.

F. annosus was isolated from 60 percent of the roots of the check stumps and 15 percent of the roots from fluoride-treated stumps after 6 months. Its farthest extension was 18 inches from the root collar. It was absent from roots of the borax-treated stumps. P. gigantea was isolated from a few roots from check and fluoride-treated stumps, but always proximally to F. annosus. Trichoderma spp. and other microorganisms were isolated both proximally and distally to F. annosus, indicating root invasion from both stump and soil. Roots from borax-treated stumps were invaded by blue stain fungi, principally a Verticicladiella sp., which entered the roots from the soil.

All roots sampled at this period, especially those from borax-treated stumps, were heavily damaged by feeding of adults and larvae of Hylobius pales (Herbst.).

After 9 months, F. annosus was not recovered from fluoride-treated stumps, and had almost disappeared from the check stumps. In the check stumps it had been replaced almost equally by Trichoderma spp. and P. gigantea. In fluoride-treated stumps, Trichoderma spp. were dominant, often the only fungi colonizing the stumps. P. gigantea was isolated only occasionally. Fusarium moniliforme, blue stain fungi, Trichoderma spp., and occasionally P. gigantea were isolated from borax-treated stumps.

The percentage of roots of check stumps in which F. annosus was recovered decreased considerably between the 6- and 9-month sampling dates.

A small increase was shown in roots from fluoride-treated stumps. P. gigantea was isolated more frequently from roots of check and fluoride-treated stumps. Little change was noted in roots from borax-treated stumps.

At the 12-month sampling period, a further reduction in percentage of roots of check stumps colonized by F. annosus was noted, whereas colonization of roots of fluoride-treated stumps increased again. F. annosus was isolated from three roots from borax-treated stumps. In all three roots the fungus was present in relatively short segments, 6 to 8 feet from the root collar, which indicates direct root infection.

At the 12-month sampling period, P. gigantea was present in 77 percent of the roots from check stumps. Many of the roots were so completely decayed by this fungus that they were difficult to dig up without breaking. A small increase in P. gigantea was noted in roots of fluoride- and borax-treated stumps.

Little change was noted in the frequency with which F. annosus was isolated between 12 and 18 months for all treatments. The percentage of roots yielding P. gigantea and Trichoderma spp. declined considerably in roots of check and fluoride-treated stumps. Bacteria and miscellaneous fungi were recovered from most of the roots. Some roots were so badly decomposed at this time that little remained except the bark. Roots from the borax-treated stumps were much less decayed, probably due to heavy blue stain. However, many roots were riddled with larval tunnels of H. pales.

For the most part, roots from which F. annosus was isolated had but few other fungi present after 18 months. These roots were less deteriorated than those in which P. gigantea was found.

DISCUSSION

The results of this study suggest that the failure of ammonium fluoride to give long-term control in the original tests was due to improper early evaluation. In the present study, samples taken from the top 2-3 inches of the stump 3 months following treatment would have shown only a small percentage of the stumps to be infected by F. annosus as most of this area was colonized by Trichoderma spp. at this time. F. annosus was detected only by systematically sampling the stump to depths not yet penetrated by

Trichoderma. Better results would have been obtained 1 month after treatment before Trichoderma spp. became dominant in the upper portion of the stump.

Some preliminary laboratory studies showed that 0.5 percent ammonium fluoride in malt-extract agar prevented both mycelial growth and conidial and basidiospore germination of F. annosus. However, even a 4 percent solution did not prevent stump infection after only 1 month, although some decrease was noted over the check. Previously Berry (1965) reported that 5 percent ammonium fluoride gave excellent stump protection against infection by F. annosus based on isolation from the top 2 inches of the stump 3 months after treatment. Berry also indicated that urea and ammate both gave control equal to ammonium fluoride and also stimulated growth of Trichoderma spp. and P. gigantea.

It is possible that urea, which was reported by Berry (1965), Sinclair (1964), and Morris (1962) to give excellent control, may also stimulate the growth of Trichoderma spp. to the degree that F. annosus would not be recovered using the usual evaluation techniques. Indeed, Morris (1965) later indicated that after 3 years, urea was only about 45-60 percent as effective as reported after 3 months. This report was based on presence of sporophores on the stumps and should therefore be considered a conservative estimate of the percentage of stumps with F. annosus. More work needs to be done with long-term evaluation of this material, which is widely recommended for use as a stump protectant in the northeastern United States.

The percentage of roots of fluoride-treated stumps from which F. annosus was isolated was higher than in roots of check stumps after 18 months. This was attributed primarily to replacement of F. annosus in the check stumps and roots by P. gigantea. The latter fungus was either unable to compete well with the high level of Trichoderma spp. in the fluoride-treated stumps or was less tolerant to ammonium fluoride. Unpublished research by the author showed that P. gigantea can replace F. annosus in root wood even if F. annosus is the primary colonizer. Several species of Trichoderma tested so far have been unable to do this. Results of the present study indicate that Trichoderma follows behind the actively growing front of F. annosus in the stump while P. gigantea is able to overtake and completely replace F. annosus. This is true even though growth rate of Trichoderma spp. in culture is considerably faster than either F.

annosus or P. gigantea. Similar findings of the relative competitive abilities of F. annosus, P. gigantea, and Trichoderma spp. were reported earlier by Kuhlman and Hendrix (1964).

These results, as well as observations from other studies, substantiate the work of Rishbeth (1959), Boyce (1966), Kuhlman and Hendrix (1964), and others who showed P. gigantea to be an effective competitor of F. annosus. If the natural inoculum of this fungus could be increased, as proposed by Boyce (1966), it might prevent F. annosus from becoming a more serious hazard in pine plantations.

Borax applied to the stump surface significantly reduced colonization by all organisms, including F. annosus, for almost 6 months. After this time, Trichoderma spp., Fusarium moniliforme, bacteria, and blue stain fungi were the primary stump colonizers. Root colonization was primarily from the soil and for the most part was by Verticicladiella sp., Graphium sp., and bacteria. A Candida sp. was commonly isolated from frass of H. pales larval tunnels.

Although the study was not carried through to the logical conclusion of checking for infection in the residual stand after an appropriate period, the borate-treated stumps and roots after 18 months were so deteriorated by feeding of H. pales larvae and so completely colonized by other microorganisms that further infection by F. annosus was unlikely. On this basis, borax appears to be an effective stump treatment.

At least four and possibly seven instances of direct root infection by F. annosus were recorded. None were definitely correlated with feeding wounds of H. pales adults but such wounds would make ideal infection courts. Hendrix and Kuhlman (1964) have demonstrated direct root infection by spores of F. annosus moving downward through the soil, germinating on wounds, and initiating infection. Although the frequency of direct root infection recorded in this study was low, this factor must be considered in evaluating the effectiveness of stump protectants.

F. annosus was isolated from several roots to a distance of almost 6 feet from the root collar after 12 months. This is almost twice the growth rate of roots found by Rishbeth (1951) for this fungus in England, and somewhat greater than the 53 inches in 10 months reported by Kuhlman and Hendrix (1964).

The following conclusions can be drawn from this study:

1. Ammonium fluoride is not an effective stump protectant against *F. annosus*.

2. Borax is an effective stump treatment.

3. Stump treatments which stimulate heavy mold growth must be evaluated systematically by isolating from several levels in the stump to insure the molds do not mask *F. annosus*, thus giving a false impression of control.

4. *P. gigantea* is an effective natural competitor of *F. annosus*. *Trichoderma* spp. do not seem to be nearly as effective.

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INOCULATION OF PINE AND SPRUCE SEEDLINGS WITH CONIDIA OF *FOMES ANNOSUS* (FR.) CKE.

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Mostly it has been taken for granted that conidia of *Fomes annosus*, sometimes named *Heterobasidium annosum* Bref. or *Oedocephalum lineatum* Bakshi, are of limited importance for the dissemination of the fungus (Ferdinandsen and Jørgensen, 1938-1939; Lagerberg, 1958) or are negligible in practice (Rishbeth, 1951; Peace, 1962). Morris and Knox (1962) studied stumps in February of loblolly pine from a thinning the preceding November, and the high frequency of conidia on the incubated discs gave them an occasion to suppose, that conidia of *Fomes annosus* play a more pronounced role as infectious agents than is commonly considered.

As a certain support to the opinion of the latter authors, some experience from a preliminary experiment could be reported, using conidia of *Fomes annosus* as inocula on small plants of *Pinus silvestris* L. and *Picea abies* Karst.

METHODS AND RESULTS

Seedlings of Scots pine and Norway spruce, germinated under sterile conditions, were planted in glass beakers in accordance to a method elaborated by Lundeberg (1960). This method was however modified thus, that the beakers were not filled up with the nutrient solution or supplied with aeration, but the inside of the glass walls were covered with double sheets of Whatman No. 3 filter paper and the nutrient solution was supplied only to a third of the whole volume. No extra aeration was arranged. The roots of the seedlings were inserted between the paper and the wall of the beaker and in a good contact to the capillarily moistened paper. In each beaker 11 seedlings were planted. Three series including pine and spruce were treated; one with varying concentrations of NH_4NO_3, one with differing pH-values and one with sucrose added.

After 6 months of growth in a climate chamber the plants were inoculated with a spore suspension of Fomes annosus containing 500,000 conidia/ml. sterile distilled water. With an injection needle 0.5 ml. was put in contact to the roots at opposite sides of the beaker and without causing damage to the roots. In another series Trichoderma viride Fr. was included but shall not be reported in this connection. Reading off was performed once a week and symptoms of wilting and development of mycelia were observed.

The first symptoms appeared 2-3 weeks after inoculation as discoloured needles. After 6-10 weeks white mycelia were observed on the adventive roots and the wilting symptoms were more pronounced. There appeared no principally differences of the symptoms or mycelial development between pine and spruce.

A couple of samples were removed from the roots and transferred to malt agar slant cultures and without any exception Fomes annosus was recovered. Thus the postulates, demanded by Koch (in Garrett, 1956) to complete a disease investigation, were realized: 1. constant association of the organism with the disease, 2. isolation of the organism in pure culture, 3. reproduction of the disease by inoculation with the pure culture of the organism, 4. re-isolation of the organism from the diseased host.

From the experiment performed under the special conditions reported above, it is obvious that the inoculation has succeeded with conidia of Fomes annosus. This is nevertheless remarkable, because inoculation was achieved on un-injured roots in an active growing phase and well supplied

with nutrient solution. So far as is known, this is the first time the neglected conidia of Fomes annosus proved virulent by establishing wilt symptoms of forest tree plants, identic with those caused by the fungus in Scots pine. Wallis (1961) reports that under field conditions inoculation with basidiospores succeeds only when the roots are injuried and the surface of the actual root-piece sterilized. Analogous experiences are published by Rishbeth (1951) with inoculation experiments on young trees of Scots pine.

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PROTECTION AGAINST ATTACKS OF FOMES ANNOSUS ON CONIFEROUS SEEDLINGS BY A MYCORRHIZAL FUNGUS

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Eighty-five isolates of soil fungi, most of which may be characterized as mycorrhizal fungi, were included in a screening test against the root-rot fungus Fomes annosus. It was arrested by 40% of the isolates tested and an unoccupied, bright and measurable zone then divided the two microorganisms. Several different types of influence were observed and interpreted. A wide antagonistic spectrum was demonstrated both within and between the species. One isolate of the mycorrhizal fungus Boletus bovinus, one of B. variegatus and one from roots of Monotropa hypopitys inhibited F. annosus most strongly. The inhibition established was of a fungistatic type.

In order to widen the knowledge of the variation in sensitivity, 85 isolates of F. annosus from field trials in Norway spruce were selected and screened against three strongly active mycorrhizal fungi. As a rule all root-rot isolates were highly sensitive to all three antagonists. Only three exceptions were obtained.

The same kind of pronounced reaction to toxins was demonstrated when F. annosus was grown in nutrient solutions precultivated with some active

mycorrhizal fungi. Finally the importance of inoculation time on antagonism was studied on solid media. A strong correlation exists between time advantage and the toxic level obtained.

Based on these experiences some experiments with a host plant incorporated should be mentioned briefly.

The very antagonistic fungus Boletus bovinus could easily be grown on a medium of barley and water, which could be used as a hardy inoculum. The technique of screening seedlings of pine and spruce for protection against Fomes annosus was as follows (Figure 1): A sterile layer of perlite about 5 mm. thick was placed in seed boxes of foamplastic 250 x 125 x 120 mm. On this, a layer of autoclaved barley was applied, covering the box area. Onto this was placed 30-mm. layer of perlite, a layer of inoculated barley with Boletus, and finally another 25-mm. layer of perlite. On the surface of the perlite the 100 seeds were sown. The seeds were covered with perlite, irrigated and left under glass in artificial light and a day/night temperature of 25/15° C. As it was possible to grow Fomes on barley also (150 g. barley, 200 g. water), 16 different combinations with barley, Boletus bovinus, Fomes annosus and controls were possible. Of special interest were those where B. bovinus was the only inoculum, where B. bovinus was superior to a layer of F. annosus and where F. annosus was solely inoculated.

In all experiments with F. annosus as the only inoculum, the seedlings died. The plants were usually killed after 4 to 5 weeks when two layers of Fomes-inoculated barley were applied at different levels in the boxes. A pronounced difference could be noticed between Norway spruce and Scots pine. Of 25 spruce provenances and progenies from crossings, only 5 had a few living plants after 3 months. In pine 11 of 20 provenances had a relative number of surviving plants of 50 or more compared with the controls.

When B. bovinus was introduced as the only inoculum and as a two-layer system, the plants were often weakened and even killed. With only a bottom-layer of the mycorrhizal fungus, the plants developed luxuriantly with a high percentage of surviving individuals, rapid growth, and a deep green color. The height of spruce after 8 weeks was roughly twice that of the controls.

The combination of a bottom layer of F. annosus and a top layer of B. bovinus always gave protection from F. annosus. This protection was,

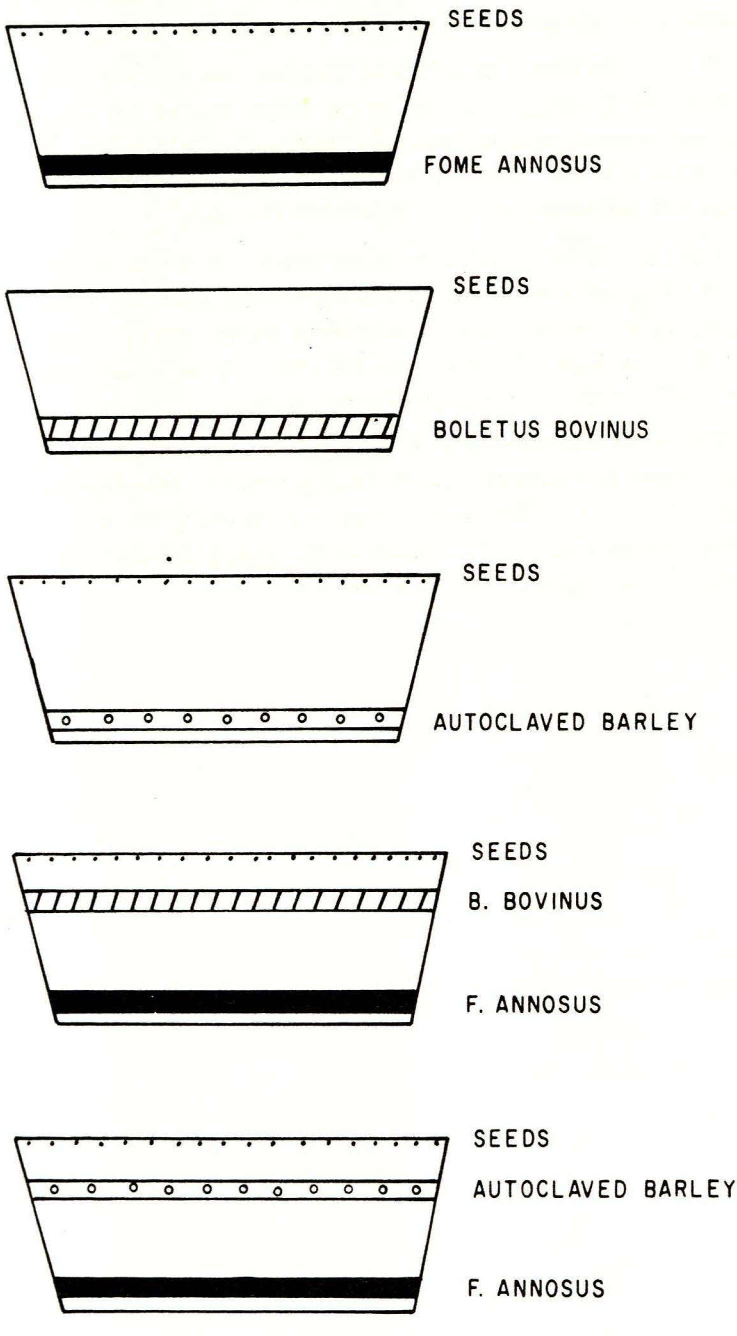

Figure 1.--Different types of treatment in the seed boxes

however, not complete but ranged from 0 to 88% of the controls in spruce. In Scots pine, the protective effect was smaller but quite observable.

Finally, it should be mentioned that the combination with barley as the top layer and F. annosus applied in the bottom delayed the killing of the seedlings compared with the two-layer combination with only F. annosus. The combination with two layers of autoclaved barley had no fatal effect on the plants, but decreased the percentage of germination.

Finally a comparative test was performed with spruce seedlings treated differently. One combination included seedlings grown in contact with B. bovinus, one in contact with autoclaved barley, and in the third case controls in perlite. After 7 weeks the seedlings were inoculated individually with F. annosus growing on barley.

The protection exerted by B. bovinus was complete during the experiment time as shown in Figure 2. The decreasing vitality of untreated controls and plants in contact with pure barley is principally the same. The rapid killing of seedlings when two layers of F. annosus are applied is represented in Figure 2 for comparison.

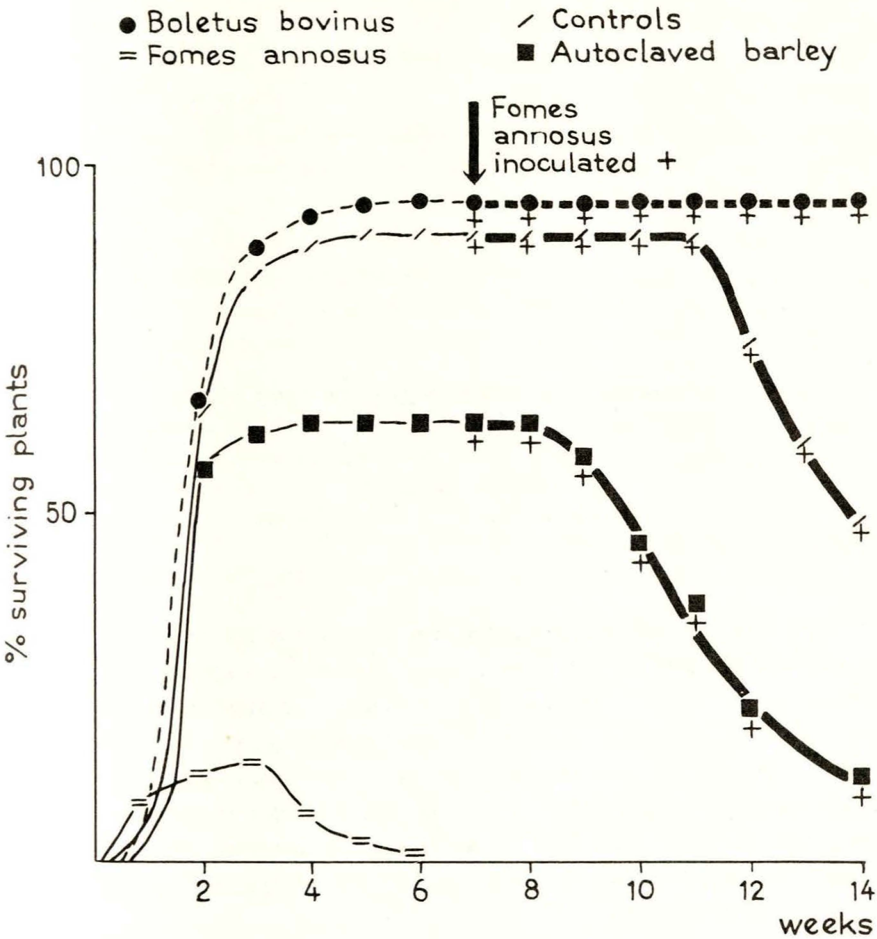

Figure 2.--Protection of Norway spruce seedlings against Fomes annosus by Boletus bovinus

SOME ASPECTS OF METABOLIC STUDIES OF *FOMES ANNOSUS*

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The physical and chemical conditions influencing the growing mycelium in the wood are difficult to investigate, as also are their metabolic effects on the fungus. *Fomes annosus* is usually the pioneer that requires fresh wood not previously attacked by other competing fungi (cf. Shigo, 1967). Even so, the survival and progress of the fungus mycelium implies a great capacity for adaptation. Also the fact that many species are suitable for attack by Fomes shows that its physiological tolerance spectrum is very broad. Therefore it does not seem easy to find specific physiological qualities of this fungus.

The pronounced adaptability of the growing mycelium involves a high capacity for changing the metabolism to suit the conditions. For example the variations in oxygen and carbon dioxide pressure or in humidity of the attacked wood may be very great, as also in the concentration and nature of phenolic substances. The resistance shown by *e.g.* sapwood of *Pinus* species results partly from the fact that host and fungus influence one another. Phenol and resin production induced in the tree by fungal attack serves as an inducer of enzymes and metabolic changes, making the fungus capable of resisting and modifying these substances. This has been suggested by Shain (1967) and others.

It was shown by Fåhraeus (1962) that several aromatic compounds having a stimulatory effect on fungal growth in many cases also increased the laccase activity. Bega and Tarry (1966) reported increased growth rate and sporulation in the presence of resins. The participation of phenol oxidases in terminal respiration has been discussed for many years. The accumulation of reduced NAD during the glucolysis must be prevented by the regeneration of NAD. The reoxidation is probably possible in several ways. Under certain conditions, especially when anaerobiosis is accompanied by laccase-inducing phenols, the presence of laccase makes possible a reoxidation and is thus involved in the electron transport system and even in the ATP synthesis (Fåhraeus, 1962).

The decomposition of lignin by Fomes annosus gives rise to phenols which have to be detoxified. The importance of laccase in this respect has been thoroughly discussed by Gadd (1957), Lyr (1965), Rösch (1965) and others.

Lyr (1957) has suggested a connection between the synthesis of laccase and the function of the tricarboxylic cycle, since most of the inducing substances could influence the reactions in the cycle and the formation of substances derived from its components. The importance of blocks in the citric acid cycle and the cytochrome-cytochrome oxidase system may consequently be of interest in this respect. Such blocks are possibly caused by reduced oxygen supply, by high concentration of carbon dioxide or by phenols, which act as chelating agents or are capable of denaturing proteins. Depressed respiration results in the accumulation of pyruvic acid, oxalo-acetic acid (carbon dioxide fixation) or alcohol, which has been proved for many microorganisms. Negrutski (1961) observed an accumulation of alcohol in Fomes cultures as a result of anaerobic conditions. Preliminary results in our laboratory indicate an increased content of keto acids in unaerated culture media with Fomes annosus as the length of incubation is increased.

The knowledge of the functions of phenoloxidases is still very fragmentary. However, it seems very likely that they are important for the adaptability of the fungus in the host. One of the aims in the Fomes studies in our laboratory is to observe the effect of lignin metabolites and other related compounds added to an otherwise defined medium, when the conditions of aeration are varied. Preliminary experiments have shown that in a medium with spruce wood powder as the only organic component the laccase activity becomes very marked and is strongly increased by the

addition of 2,5-xylidine (Fåhraeus, 1962). However, if the wood powder is replaced by pure cellulose or xylan, no laccase activity is obtained, although the cellulase and xylanase activity are still observed. In our experiments, guaiacol was chosen as one of the substrates for the laccase test. According to Rösch (1965) this substance is not attacked by tyrosinase and might be comparable with coniferyl alcohol in its chemical configuration and thus with some of the natural substrates of laccase in the wood. The pH optimum of the laccase activity on guaiacol is 4.8, while that of the related o-diphenol pyrocatechol is 4.2. At higher temperatures than the optimum (40° C.) for the enzyme, the process of denaturation is accelerated. At 60° C. the activity is stopped within a few minutes but can be restored even after one hour at this temperature, by lowering the temperature to 25° C. This is not possible at higher temperatures. Thus a reversible inactivation of the enzyme protein seems to occur within a limited temperature interval. Work is in progress to purify and characterise the phenol oxidase obtained in the wood-meal medium. Among methods used in these studies is isoelectric separation, a method dependent only on the electric charges possessed by the protein. This might increase the possibilities of finding differences between the enzymes induced under different conditions.

There are very few reports concerning the respiration of cell-free preparations of Basidiomycetes. This may depend partly on the difficulties of obtaining cells of equal age and viability. Casselton (1966) studied the oxidation of glucose by the Embden-Meyerhof and the pentose phosphate pathways for Polyporus brumalis. Lactarius torminosus was investigated in the same respect by Meloche (1962). No such work has been done with Fomes annosus, although there is reason to believe that the energy-yielding systems may be very flexible, depending on the variations of the ecological conditions. Even though no direct conclusions can be drawn about the living organism from the study of isolated systems, this method is useful for comparing the effect of various inhibitors. The inhibitors may be of phenolic type, produced by the fungal metabolism or by the host tree. Antibiotic substances from competing fungi may interfere with particular reactions, as also the amounts of oxygen and carbon dioxide present.

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DISTRIBUTION OF *FOMES ANNOSUS* SPORES THROUGH THE AIR IN FINLAND (A PRELIMINARY REPORT)

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The purpose of this investigation is to obtain a picture of the aerial distribution of *Fomes annosus* in Finland. The results reported here are based on spore sampling on 6 open observation areas and in one infected spruce stand. These samplings were carried out from the beginning of June until the end of December 1967.

MATERIAL AND METHODS

The open observation areas were the following airports: Ivalo (68° 36' North, 27° 25' East), Oulu (64° 56' North, 25° 22' East), Jyväskylä (62° 24' North, 25° 40' East), Turku (60° 31' North, 22° 16' East), and Lappeenranta (61° 03' North, 28° 09' East) besides the field in Viikki (60° 13' North, 25° 02' East). The infected forest was also in Viikki.

Picea abies cross-sections 2 cm thick with a diameter of about 14 cm were used as a growing base. In order to ensure reasonable accuracy two kinds of agar plates were used besides the cross-sections: H = Hodges (1966) and K = Kuhlman & Hendrix (1962). The first contains, inter alia,

penicillin and PCNB, the second streptomycin and PCNB. Both cross-sections and agar plates were kept at room temperature 12-14 days before miscroscopical investigation.

The Fomes annosus conidiophores were identified on the cross-sections with a stereomicroscope enlarging 25 times and on the agar plates with a normal microscope enlarging 100 times.

On the open observation areas two cross-sections of Picea abies were kept open, for two and four hours, respectively. Sampling interval was one week. A pair of H and K agar plates were kept open for 5 minutes in the same places, and another pair for 10 minutes.

Daytime was used for these samplings except for some night observations (see Figures 1-3). In addition, in one of the study areas (the infected forest) a special study was made of the effect of the hours of the day on the amount of Fomes annosus spores in the air.

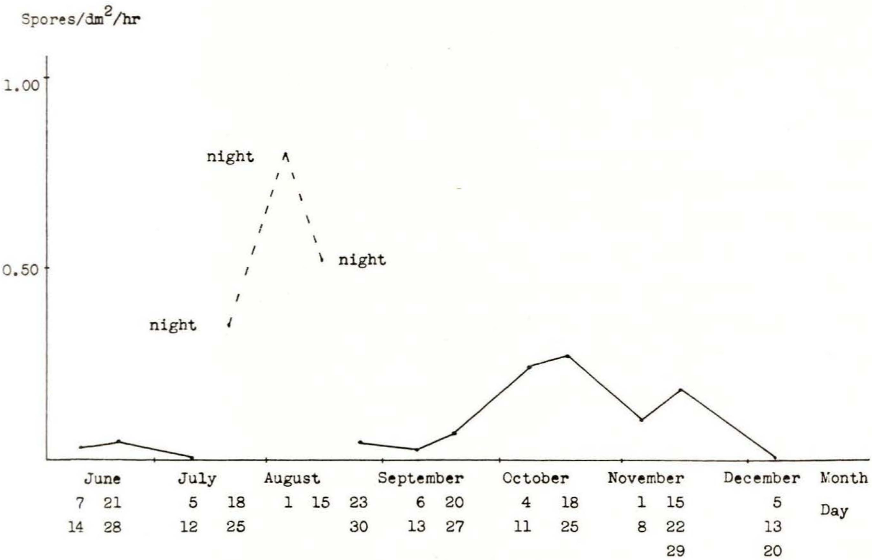

Figure 1.--Fomes annosus spores/dm.^{2}/hr.; South Finland; open observations areas; cross-section of Picea abies

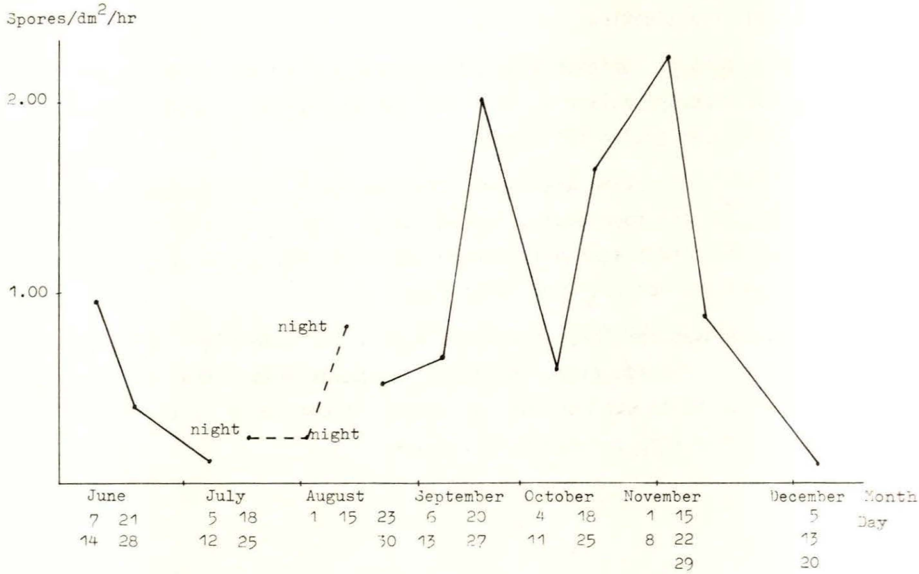

Figure 2.--Fomes annosus spores/dm.^{2}/hr.; South Finland (Viikki); infected forest; cross-sections of Picea abies

RESULTS

According to an earlier study (Kallio, 1967) the first Fomes annosus spores were in the air on March 29 in Viikki. At the time there was an 8 cm. layer of snow. On the other hand there were no Fomes annosus spores in the air during the whole survey in Ivalo and only one was trapped from Oulu. These findings support earlier results of the small role of Fomes annosus in northern Scandinavian forests. The following figures refer only to South Finland from Jyväskylä to Viikki.

The Open Areas

Figure 1 represents the settling of the spores of Fomes annosus in South Finland on the open observation areas counted from the cross-sections of Picea abies. The numbers of spores in the air was quite low during June-September with a clear maximum in late autumn. Spore numbers at night

were surprisingly high, many times higher than day-counts. They show that in Finnish conditions night seems to be the time when the Fomes annosus spores settle down.

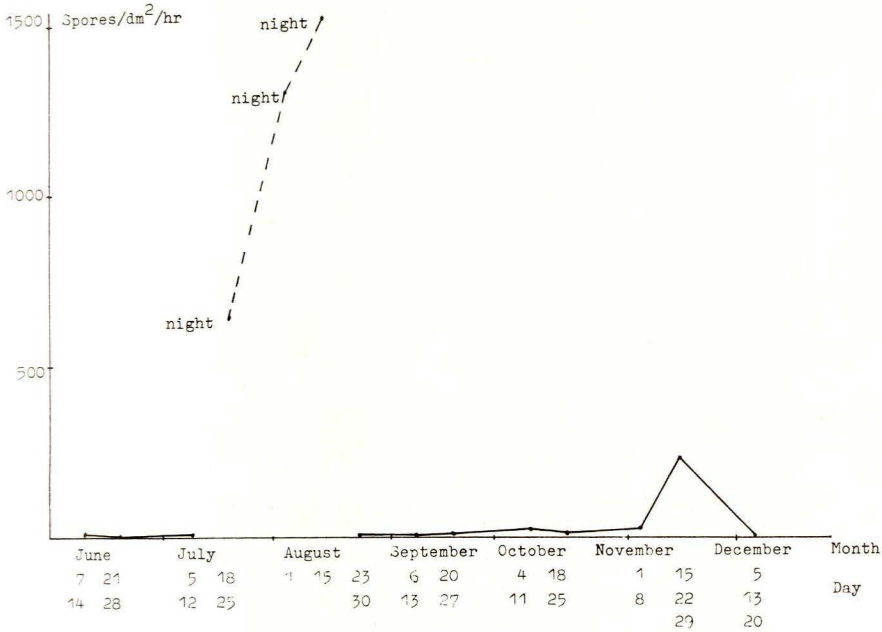

Figure 3.--Fomes annosus spores/dm.^{2}/hr.; South Finland (Viikki), infected forest; H and K agar plates

Forest

Figures 2 and 3 present the results from the infected forest in Viikki. The difference between the results from the cross-sections and the H and K agar plates is considerable but has always the same trend, a maximum in late autumn. The large amount of spores settling down at nighttime at the end of July and the beginning of August should also be noted. The maximum was reached on August 15, about 1500 spores/dm.^{2}/hr.

Time of the Day

To obtain more precise information from the spore counts during the different hours of the day Figure 4 was drawn. It is evident that the fall of Fomes annosus spores is greatest at night. The maximum was observed at 22 o'clock, about 1400 spores/dm.^{2}/hr.

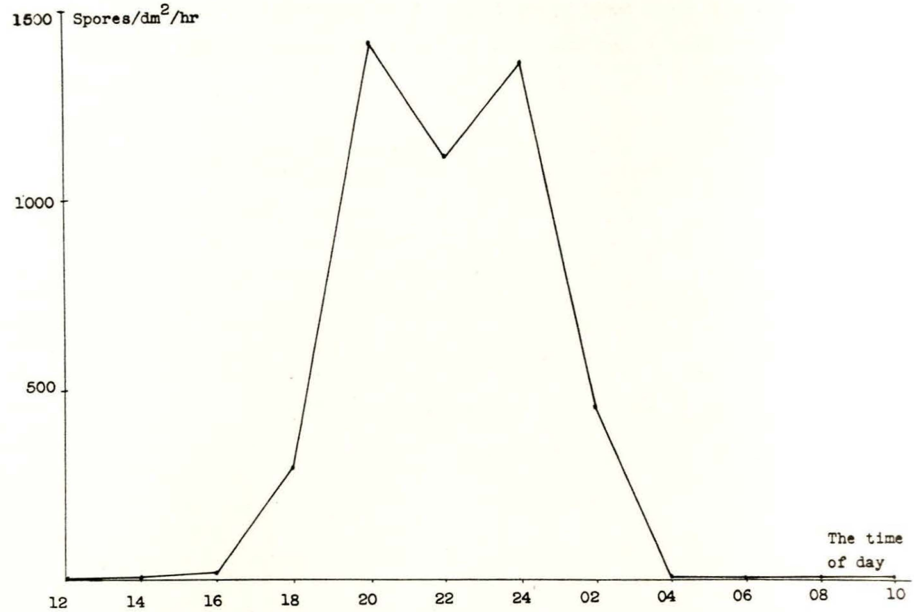

Figure 4.--Fomes annosus spores/dm.^{2}/hr. during 24-hour period (July 4-5, 11-12, 18-19, and 25-26, 1967); South Finland (Viikki); infected forest; H and K agar plates

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REGENERATION OF PINE ON FOMES ANNOSUS-INFESTED SITES IN THE SOUTHEASTERN UNITED STATES

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European experience has indicated that limited Fomes annosus (Fr.) Karst. infection in the first rotation may increase in second rotation conifer stands to more than 50% of the stand (Low and Gladman, 1960). Earlier reports from the southeastern United States focused attention on seedling mortality due to F. annosus (Driver and Ginns, 1964; Hendrix, Kuhlman, Hodges, and Ross, 1964; Ross and Hodges, 1964). Because F. annosus may remain active in southern pine stumps for many years, losses to the fungus were expected to increase dramatically as more seedling-to-stump and seedling-to-seedling contacts were made.

Replant studies were established at several localities to study the effect of F. annosus damage in second rotations on the management of pines in the southeastern United States. Several variables were tested, including the incidence of infection in transplanted seedlings vs. seedlings resulting from direct seeding; variation in susceptibility of several pine species; and the effect of site preparation on survival.

Because forest managers are confronted with the problem of regenerating infested sites, it seemed important to present the results of 5 years of observation in the Southeast. A complete answer to this problem will be available only after our study plantations have reached commercial size; however, the present trends seem more favorable for pine management than indicated previously (Driver and Ginns, 1964; Hendrix, Kuhlman, Hodges, and Ross, 1964).

PROCEDURES AND RESULTS

An initial study of 32, 1/10-acre plots was established on 4 sites on the Pisgah National Forest in western North Carolina. Thirty-two-year-old stands of white pine (*Pinus strobus* L.) had been clearcut in 1962 because of severe annosus root rot damage. Each plot was replanted in the spring of 1963 with 30 seedlings each of white pine, shortleaf pine (*P. echinata* Mill.), Virginia pine (*P. virginiana* Mill.), and Scotch pine (*P. sylvestris* L.) to test variation in susceptibility. Nine-hundred and sixty wildling white pine and Virginia pine within the infested area were marked for yearly observation. Each fall, dead seedlings were collected and cultured on an agar medium selective for *F. annosus* (Kuhlman and Hendrix, 1962). The yearly percent mortality due to *F. annosus* has remained very low for all species (Table 1). None of the species, either planted or wildling, has shown any different degree of susceptibility. The least mortality occurred among the Virginia pine wildlings. If this trend continues, it will support previous findings that wildling stock is more resistant to infection than planted stock.

Table 1.--Yearly percent mortality due to *Fomes annosus* on Pisgah National Forest, N. C., sites clearcut in 1962 because of severe *F. annosus* infestation and replanted in 1963.

Species: transplanted (T) wildling (W)	1963	1964	1965	1966	1967	Total % loss
White pine (T)	0.6[a]	0.1	0.9	0.6	0.1	2.3
Virginia pine (T)	1.4	0.8	0.4	0.8	0.1	3.5
Scotch pine (T)	0.7	0.0	0.5	0.6	0.2	2.0
Shortleaf pine (T)	1.7	0.5	0.2	0.4	0.0	2.8
White pine (W)	0.4	0.3	0.0	0.6	0.3	1.6
Virginia pine (W)	--	0.0	0.0	0.1	0.4	0.5

[a]Percent based on 960 seedlings per species.

A similar test of variation in susceptibility was established on an *F. annosus*-infested site on the Manchester State Forest near Sumter, South

Carolina, in 1964 using loblolly (P. taeda L.), slash (P. elliottii var. elliottii Engelm.), and longleaf pine (P. palustris Mill.). Four replications of 192 seedlings of each species were planted on a 6-acre site. Similar plots within each replication were direct seeded, but survival was low because of animal grazing. Survival of the transplanted stock varied considerably; however, there was little variation in losses to F. annosus among the three species (Table 2).

Table 2.--Survival of three pine species planted on a Manchester State Forest, S. C., site and the mortality due to Fomes annosus during a 4-year period.

Species	% Survival	% Loss due to F. annosus
Loblolly pine	96[a]	1.0
Longleaf pine	72	1.4
Slash pine	70	1.5

[a]Four replicates/species of 192 trees = 768 trees/species.

Study plots have also been established in plantations where seedling mortality due to F. annosus occurred, even though the previous stand had no history of F. annosus damage. One of these studies was established in a 4-year-old loblolly pine plantation near Miley, South Carolina. The plantation had previously been planted to slash pine but was clearcut in 1959 because of severe fusiform rust (Cronartium fusiforme Hedgc. & Hunt ex Cumm.). Mortality due to F. annosus in this 5-acre stand was quite evident in 1964 when the plots were established; within the 21 plots, losses ranged from 1-5%. Over the 5-year period, however, losses to F. annosus have stabilized at a very low level (Table 3).

Table 3.--Summary of mortality to Fomes annosus in a loblolly pine plantation near Miley, S. C., expressed as the percentage of the original stand lost each year over a 5-year period. Original stocking (1963) was 10,757 saplings and presently (1967) is 10,296 on 15 acres.

Cause of mortality	Age of seedlings					Total loss
	4	5	6	7	8	
Fomes annosus	1.9[a]	1.3	0.3	0.4	0.2	4.0

[a]Includes data from only 12 of 21 plots.

Another study was established in a 3-year-old slash pine plantation near St. Augustine, Florida. A natural, mixed longleaf pine and hardwood stand was clearcut in 1960, the site was prepared by chaining and harrowing in 1961, and the slash pines were planted in 1962 at a spacing of approximately 6 x 8 feet (900/acre). By 1964, mortality due to F. annosus was evident in several locations throughout the 204-acre plantation. A 1964 survey of 50 acres of this plantation showed that overall only 1.0% of the seedlings had been killed by F. annosus and an additional 2.1% were infected but still living. Data from five 1/4-acre plots established in some of the more heavily infested areas are summarized in Table 4. These data also indicate that over the 4-year period, losses caused by F. annosus have stabilized. Many seedlings which exhibited conks in 1964 showed no symptoms of annosus root rot in 1967.

Table 4.--Summary of mortality caused by Fomes annosus in a slash pine plantation near St. Augustine, Fla., expressed as the percentage of the original plot infected or lost each year over a 4-year period.

Plot No.	Age of seedlings (years)							
	3		4		5		6	
	Infected[a]	Dead	Infected	Dead	Infected	Dead	Infected	Dead
1	17.8	4.0	4.3	0	3.0	0	1.0	0
2	3.7	1.0	4.8	0	2.0	0	1.0	0
3	5.5	5.5	5.9	1.7	1.6	0	2.3	0
4	11.8	5.8	7.3	0	0	0	0	0
5	3.0	3.0	3.1	0	0	0	0	0

[a]Infected but not dead, determined by the presence of a F. annosus sporophore.

Site preparation prior to planting has become more intensive in many areas of the Southeast. Because F. annosus survives saprophytically in the roots and stump body, any site preparation that breaks up the stumps and roots should increase chances for replacement of F. annosus by other fungi and thereby reduce the chance of new infection. To determine what effect site preparation has on the survival of seedlings planted in areas severely infested with F. annosus, a study was established near Summerville, S. C., in 1965. The treatments are: (a) no site preparation; (b) subsoil to 12 inches in two directions; (c) disk to 6 inches in one direction; (d) remove stumps with a root rake; (e) no site preparation, natural reproduction allowed to develop. The entire study area lay fallow for one year following

the site preparation treatments. Each one-acre plot in treatments (a)-(d) have 3 subtreatments; machine-planted seedlings, hand-planted seedlings, and direct seeding. During the first 2 years, losses have been so low (0.05%) in all plots that thus far this study can be used only to show that in some situations annosus root rot may not be a problem in young reproduction.

Natural pine reproduction often appears in old F. annosus centers in the southeastern United States. Natural reproduction was used to establish some treatments we have described on the Pisgah National Forest, N. C., and at Summerville, S. C. Because F. annosus is known to persist in decaying stumps for many years (Low and Gladman, 1960; Rishbeth, 1951), it appeared that either natural reproduction escaped the disease or was less susceptible than planted stock. One large center in the Sand Hills State Forest near Cheraw, S. C., had some reproduction 9 years old, 18 feet tall, and with a d.b.h. of 4.3 inches. The oldest trees were not in the middle of the infection center, which may indicate that more than one center coalesced. Stumps in this center were sampled to determine how many were still infested. F. annosus was recovered only from stumps at the edge of the center. Only 20% of the stumps sampled contained F. annosus. A visual estimate of the percent decay for each stump was made, and 12 of 17 stumps with F. annosus were less than 50% decomposed. In contrast, 90 of 100 of the remaining stumps were 90% or more decomposed.

Similarly, in study plots established in 1959 to follow rate of spread of F. annosus, some natural reproduction is 9 to 12 years old and 15 to 20 feet tall. In the 1/10-acre plots, F. annosus was recovered from only 4% at Sumter, S. C., and from only 15% of the stumps at Manning, S. C. Stumps that are older than 8 years were completely decomposed except for bark fragments.

DISCUSSION

These studies of losses to F. annosus in young reproduction indicate considerable variation in mortality at different sites. At Miley, S. C., and St. Augustine, Fla., mortality initially ranged up to 6%. In contrast, on a 20-acre site at Summerville, S. C., losses have amounted to 0.05% of the stand in 2 years. Trees in some of the studies are now 15 feet tall so that contacts between neighboring tree roots should have occurred, but losses have not accelerated. Losses have been so low that there has been no indication of any variation in susceptibility among the seven species of

pine tested. Natural regeneration and direct seeded plots have sustained little mortality. Previously (Hendrix, Kuhlman, Hodges, and Ross, 1964), we theorized this reduced loss in natural regeneration might be due to an initial high loss of the most susceptible individuals, but there was no high mortality rate initially in direct seeded plots at Summerville. The reduced losses might better be explained as disease escape.

The presence of natural regeneration in old annosus centers does not in itself indicate the rapid depletion of the fungus. However, pine stumps in the Southeast decompose so rapidly that in several instances there were only bark fragments remaining after 8 years. In the southeastern United States, pine stumps are relatively small and contain little heartwood because of short rotations, generally near 30 years. Termite and root weevil activity is vigorous and warm soil temperatures and adequate soil moisture provide optimum growth conditions for the wood-decomposing fungi. These factors would seem to indicate that F. annosus may not be an important consideration when regenerating pine plantations in the Southeast. Observations will be continued, however, until these plantations have reached commercial age so as to provide the best information for forest managers in terms of the annosus hazard to reproduction.

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URSACHEN DER INFEKTIONSBEREITSCHAFT DER FICHTE (_PICEA ABIES_ KARST) AUF BESTIMMTEN STANDORTEN

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Nach Untersuchungen von H. Zycha und F. Kató (1967) wird die Kernfäule der Fichte in Norddeutschland in erster Linie durch _Fomes annosus_, ferner durch _Armillaria mellea_ und einige andere Pilze hervorgerufen. Man hat sich bisher weit mehr mit der Ausbreitung, den Infektionswegen, den Antagonisten und der Biologie des häufigsten Erregers als mit der Disposition der Bäume für eine Infektion befasst. Da in Deutschland auf ganz bestimmten Standorten alter Waldböden regelmässig ein grosser Teil der Stämme eines Fichtenbestandes von der Wurzel aus infiziert wird, Bestände auf anderen Standorten dagegen zum grössten Teil gesund bleiben, liegt es nahe, nach einem Zusammenhang zwischen dem Ernährungszustand und der Resistenz der Fichte zu suchen.

Es ist bekannt, dass sich viele höhere Pflanzen durch Hemmstoffe bis zu einem gewissen Grade vor Infektion schützen können. Häufig gehören diese Hemmstoffe zu den Polyhydroxyphenolen oder zu den ätherischen Ölen. Solche sekundären Pflanzenstoffe werden auf bestimmten Standorten in grösseren Mengen als auf anderen, die zum gleichen Klima gehören können, produziert.

Das ist eine beim Anbau von Arzneipflanzen häufig gemachte Beobachtung. Es lag daher nahe, die Fichte nach Stoffen zu untersuchen, die das Mycelwachstum von Fomes annosus hemmen.

HEMMSTOFFE DER FICHTENRINDE

Nach H. Endres (1961) besteht die Trockensubstanz des Fichtenbastes zu etwa 17-21% aus löslichen, gerbenden Stoffen, während das Fichtenholz frei von Gerbstoffen ist. Der grösste Teil der Gerbstoffe des Bastes gehört zu Polyhydroxyphenolen aus der Gruppe der Stilbene. Sie liegen zum Teil in freier Form, zum Teil in glycosidischer Bindung vor und gehen an der Luft, aber auch in der Borke durch Oxydation und Kondensation in unlösliche, stark gerbende Phlobaphene über. Der wichtigste Vertreter der löslichen Gerbstoffe des Bastes ist Piceatannol, nach J. Cunningham, E. Haslam und R. D. Haworth (1963) ein Tetrahydroxystilben. Mit diesem Stoff ist das Pinosylvin, ein Dihydroxystilben, eng verwandt. H. Erdtman isolierte es aus Kiefernholz, und E. Rennerfelt (1950) zeigte, dass Pinosylvin die Keimung von Fomes annosus Sporen stark hemmt. Da man mit H. J. Braun (1958) die Phlobaphene der Fichtenborke als chemische Barriere für Fomes annosus betrachten kann, ergab sich die Frage, ob auch die im Bast der Fichte angereicherten löslichen Vorstufen Hemmstoffcharakter besitzen.

Im Malzagar-Plattentest zeigte sich, dass Bastscheiben, Bastmehl, der Chloroformextrakt des Bastes und auch die Polyphenol-Extrakte das Mycelwachstum von Fomes annosus hemmen. Versetzt man den Malzagar mit 0,5% eines Chloroform-Extrakt-Präparates oder mit 0,5% eines Präparates, in dem die Polyhydroxyphenole und ihre Glycoside angereichert sind, sterilisiert und beimpft die Mitte der Platte mit einem ausgestanzten Mycelstückchen, so wird das Durchmesserwachstum auf den Platten innerhalb von 8 Tagen bei 23-24° C deutlich gegenüber den Kontrollen gehemmt, ebenso die Massenentwicklung des Luftmycels. Über Einzelheiten des Verfahrens vergleiche man W. Laatsch, M. Alcubilla, G. Wenzel und H. v. Aufsess (1968).

Durch umfangreiche Untersuchungen wollen wir prüfen, um welche Hemmstoffe es sich im einzelnen handelt, und ob ihre Konzentration im Bast der Fichtenrinde in Abhängigkeit vom Standort und von dem jeweiligen Wasservorrat des Bodens stärkeren Schwankungen unterworfen ist. Es ist nicht unwahrscheinlich, dass die Fichtenwurzel nur solange Hemmstoffe in genügender Menge produziert, wie sie reichlich mit Zucker versorgt wird; denn Zucker ist Rohmaterial für die Hemmstoffproduktion. Sobald nur wenig Zucker zur Verfügung steht, weil die Photosynthese aus Mineralstoffoder

Wassermangel herabgesetzt ist, oder weil wegen eines sehr starken Stickstoffangebotes ein erheblicher Teil des Zuckers für den Aufbau von Aminosäuren und Amiden verbraucht wird, könnte die Hemmstoffkonzentration im Bast niedriger als bei einem normalen Ernährungszustande sein.

Sollte diese Arbeitshypothese zutreffen, so lässt sich die starke Anfälligkeit der Fichte auf bestimmten Standorten besser verstehen.

STANDORTE MIT GERINGER RESISTENZ DER FICHTE

In Süddeutschland hat E. Rohmeder (1937) das gehäufte Auftreten der Stammfäule auf bestimmten Standorten genauer beschrieben, und aus Norddeutschland verdanken wir H. Zycha und F. Kato (1967) entsprechende Untersuchungen. Nach diesen Arbeiten und eigenen Beobachtungen sind Fichtenbestände in Deutschland stark durch Fomes annosus gefährdet:

1. wenn ihr Wurzelsystem flach streicht und der Boden periodisch stark austrocknet;

2. wenn der Boden bis zur Oberfläche stark karbonathaltig ist, oder wenn bikarbonatreiches Hangzugwasser periodisch im durchwurzelten Bodenbereich auftritt;

3. wenn der Boden reich an Stickstoff ist (Ackeraufforstungen, Basaltlehme);

4. wenn Kombinationen der unter 1-3 genannten Standortsmerkmale auftreten, ist die Gefährdung besonders gross.

Zu 1: AUSTROCKNUNG DES DURCHWURZELTEN BODENS

Einem flachstreichenden Wurzelsystem steht nur ein geringer Vorrat an aufnehmbarem Wasser zur Verfügung. In Gebieten mit Dürreperioden im Frühling oder Sommer muss der schnelle Wasseraufbrauch zu einer starken Einschränkung der Transpiration und Photosynthese führen. Wir nehmen an, dass in solchen Perioden nicht nur der Zuckertransport zu den Wurzeln sondern auch deren Hemmstoffkonzentration stark zurückgehen können. Diese Vorstellung macht die Häufung der Kernfäuleschäden auf flach durchwurzelten, nach Süden bis Westen geneigten Hängen oder auf manchen Pseudogleyen verständlich.

Zu 2: DER KARBONATGEHALT DES BODENS

Erschwert die Aufnahme von Kalium, Mangan und Eisen und erniedrigt damit u.U. die Zuckerproduktion; denn diese Elemente sind für den photosynthetischen Prozess unentbehrlich. Nach Untersuchungen von W. Zech (1968) sind Koniferenbestände auf kalkreichen Schotterstandorten Süddeutschlands häufig schlecht mit Kalium versorgt, weil diese Böden nur wenig Feinerde in der Raumeinheit des Bodens enthalten. Den Wurzeln ist ja praktisch nur das Kalium in der Feinerde zugänglich. Ein hoher Karbonatgehalt der Feinerde schliesst zudem einen hohen Kaliumvorrat aus und macht das vorhandene Kalium wegen des K/Ca-Antagonismus schwer aufnehmbar.

Der in Süddeutschland auf Karbonatböden weit verbreitete Manganmangel der Fichte wurde von W. Zech (1968) und von K. Kreutzer in noch nicht veröffentlichten Arbeiten nachgewiesen. Ältere Fichtenbestände mit starkem Manganmangel sind an einer Chlorose der jüngsten Nadeln erkennbar, die nicht etwa wie beim Kaliummangel nur die Nadelspitze sondern die ganze Nadel erfasst.

Chlorotisch werden die Nadeln derjenigen jungen Triebe, welche nicht in der Nähe der Zweigspitzen sitzen sondern weiter rückwärts in Stammnähe. Die Nadeln der oberen Wipfelregion bleiben grün, weil das Mangan sich in den Spitzenregionen der Krone ebenso wie in den Astspitzen anreichert. Manganmangel und starker Rotfäulebefall sind in Süddeutschland häufig gemeinsam auf Kalkstandorten zu beobachten.

Zu 3: STICKSTOFFREICHTUM DES BODENS

Findet man in Süddeutschland unter vielen Ackeraufforstungen und in alten Waldböden, die aus Basaltverwitterung hervorgegangen sind. An solchen Standorten ruft die Wurzel- und Stammfäule häufig besonders grosse Schäden hervor. Vermutlich werden die Wurzeln durch das hohe Angebot an Mineralstickstoff gezwungen, einen grossen Teil ihrer Kohlenhydrate für den Aufbau von Aminosäuren und Amiden zur Verfügung zu stellen, so dass weder für einen reichlichen Ligninaufbau noch für die Hemmstoffproduktion genug Rohmaterial zur Verfügung steht.

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SOME INVESTIGATIONS ON *FOMES ANNOSUS* FR. CARRIED OUT IN POLAND

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Hundreds of hectares of *Pinus silvestris* stands in Poland, especially when established on abandoned farmland, are severely attacked by *Fomes annosus*. Therefore a series of investigations, coordinated by the Polish Academy of Sciences, have been undertaken to solve the problems involved here. The intention of this paper is to report on some *Fomes annosus* investigations carried out in the Chair of Forest Pathology of the College of Agriculture, Faculty of Forestry, Poznań.

THE MYCOFLORA OF THE SOIL ENVIRONMENT AND ITS RELATION TO *FOMES ANNOSUS*

Here was undertaken a trial to substitute communities of soil fungi (*sensu lato*, *i.e.* representing different parts of the soil environment) for the ecological conditions of the soil while studying the influence of those conditions on the activity of *Fomes annosus*. It was assumed for this purpose that at least two postulates be fulfilled: 1. the methods used for isolating communities of fungi should allow obtaining results sufficiently corresponding with the type of fungal communities really inhabiting the parts of the soil environment investigated, and 2. the communities of fungi obtained should be treated in a manner showing their influence on the behaviour of *Fomes annosus*.

The subjects of investigations were Pinus silvestris stands of the Forests: Zielonka, Kleka, and Laski. Pine stand I (Forest Zielonka, section 54a), established on abandoned farmland and about 25 years of age, was growing well and disease free. Pine stand II (Forest Kleka, section 107b), also established on abandoned farmland and at similar age as the first one, was growing somewhat more slowly and was heavily infested with Fomes annosus. The next eight pine stands investigated were more or less healthy. Four of them were chosen in the Forest Zielonka, which is known to be only relatively slightly attacked by Fomes annosus, and four of them in the Forest Laski, where the incidence of this pathogen seems to be rather high.

Methods

For isolating fungi from soil, the modified soil plate method (or the sand-soil method) described recently by Mańka (1964) was used.

For isolating fungi from the rhizosphere and from the roots the following procedure was used. Apparently healthy fine roots, usually not exceeding 1 mm. diameter, were collected in a sterile flask and brought to the laboratory. As soon as possible 1 g. of these were weighed. Meanwhile nine 200-ml. flasks were prepared, each of them containing 70 ml. sterile water, and in addition 30 g. of fine sterile quartz sand. The weighed roots were shaken by turn in each of these flasks for two minutes, rinsed in sterile water, dried superficially with aid of sterile blotting paper, cut into 5 mm. long fragments, and plated on Hagem-Melin agar of the following composition: glucose, 20 g.; malt extract, 5 g.; KH_2PO_4, 1 g.; NH_4-tartrate, 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; Fe citr. 1% sol., 0.5 ml.; $ZnSO_4$ 1:500 H_2O sol., 0.5 ml.; B_1, 50 gamma; agar, 15 g.; dist. H_2O, 1000 ml. Incubation period was 6-12 days at 23° C. The resulting fungal colonies were transferred and determined.

The rhizosphere fungi were isolated on Martin-Johnson's agar (Johnson, 1957) plus 0.5% soil extract (prepared by keeping 500 g. of soil in 1500 ml. of tap water for 12 hours at room temperature with subsequent filtering through a bacterial filter). Drops of the water suspension resulting from washing of roots in flasks No. 1, No. 4, and No. 9, were put on the solidified medium surface, distributed on it by the aid of delta-shaped glass-rods, and incubated at 23° C. for 6 days. This technique encloses

elements elaborated by Harley and Waid (1955) and by E. S. Tetter (Timiriazew Agricultural College at Moscow). The fungal colonies were counted, transferred and determined.

For isolating fungi from litter, samples of litter were taken from several points in the tree stand, mixed, 5 g. of this mixture put into a flask containing 150 g. of fine sterile quartz sand, and mixed by heavy shaking for 10 minutes. The resulting mixture was in turn transferred in portions of 30 $mm.^3$ into Petri dishes and pored with Martin-Johnson's agar, while gently rotating the plates. Resulting fungal colonies were treated as mentioned above.

The communities of fungi isolated from the soil, rhizophere and other parts of the soil environment were then arranged into so-called biotic series to show the function of these communities in relation to *Fomes annosus*. The procedure was as following: Inocula of each of the fungus species belonging to a given fungal community were individually put on the surface of potato-glucose agar near the inoculum of *Fomes annosus*. The distance between the inocula of the soil fungus and pathogen was about 2 cm. All inocula consisted of 3 mm. discs taken from cultures, except the inocula of *Penicillium* spp. where drops of spore suspension were used. After 10-14 days incubation at 23° C., the cultures were examined on the basis of the following estimating scale of the mutual influence of the two-fungi, where "C" = a species belonging to the fungal community, and "F" = *Fomes annosus*.

Description of the situation in the two-fungi culture	Estimation
Both colonies meet along a straight line	0
Colony C meets colony F along a slightly curved line so that it surrounds less than 1/3 of colony F	+1
Colony C meets colony F along a curved line so that it surrounds at least 1/3 but less than 1/2 of the colony F	+2
Colony C meets colony F along a curved line so that it surrounds at least 1/2 but less than 2/3 of the colony F	+3
Colony C meets colony F along a curved line so that it surrounds 2/3 or more of the colony F	+4
Each mm. of the inhibition zone between the two colonies, when caused by the action of the colony C	+1
Colony F at least 1/3 but less than 1/2 smaller than its check-colony grown individually on another Petri-dish	+1
Colony F at least 1/2 but less than 2/3 smaller than its check-colony	+2

Description of the situation in the two-fungi culture	Estimation
Colony F at least 2/3 smaller than its check-colony but not completely undeveloped	+3
Colony F completely undeveloped	+4

If the situations in the cultures are reversed in relation to those quoted above, the estimates are the same value but negative. The relation of each of the fungus species belonging to the community to Fomes annosus is defined by the algebraic sum of estimates of its particular characters (different types of antagonistic action) involved in the system presented. Positive values of estimation indicate a negative influence on the development of Fomes annosus, negative values--a positive influence on this pathogen. The fungal communities are then arranged into the biotic series mentioned above. For this purpose about 15 species of fungi of the highest frequency within the community (representing at least 70% of colonies of the community) are placed together in a manner shown in Table 1.

Table 1.--The influence of the rhizosphere-fungi from Pinus silvestris stand II on the development of Fomes annosus

No.	Species of the fungus	Degree of biotic influence on Fomes annosus	Frequency within the community	Product of data from columns 3 and 4
1	2	3	4	5
1	Hormodendrum microsporioides	-2	44	- 88
2	Penicillium funiculosum	-2	23	- 46
3	Penicillium fellutanum	+6	22	+132
4	Penicillium lanosum	+6	20	+120
5	Penicillium sp.	-5	19	- 95
6	Penicillium jenseni	+2	15	+ 30
7	Hormodendrum elatum	-5	12	- 60
8	Cladosporium herbarum	-3	11	- 33
9	Mycelium radicis atrovirens	-2	11	- 22
10	Penicillium spinulosum	+1	10	+ 10
11	Penicillium waksmani	+5	8	+ 40
12	Absidia spinosa	+2	7	+ 14
13	Trichoderma glaucum	+7	7	+ 49
14	Penicillium steckii	-2	6	- 12
15	Mortierella vinacea	-3	5	- 15
			220	+ 24

Results

In Table 1 the idea of the biotic series is exemplified. This table shows quasi the functional structure of the community of rhizosphere fungi isolated from attacked pine stand II in relation to Fomes annosus.

The summary effect of the community of rhizosphere fungi (on Fomes annosus) presented in Table 1 is expressed numerically and totals +24. In a similar way were defined summary effects of other communities of fungi isolated from different parts of the soil environment of the pine stand I, which was healthy, and of the pine stand II, which was affected by Fomes annosus. All these summary effects, jointly with that from Table 1, are given in Table 2.

Table 2.--Summary effects on Fomes annosus of fungal communities isolated from pine stand I (healthy) and from pine stand II (affected by Fomes annosus)

Pine stand	Communities of fungi from		
	soil	rhizosphere	roots
I	+1157	+5138	+5199
II	+3561	+24	-39

The results relating to the rhizosphere- and root-fungi seem to be in accordance with the active state of health of the pine stands compared. An opposite result was obtained when the communities of soil fungi are compared, although the sampling of soil, number of replications (here 30), and the treatment of the materials from the tree-stands investigated were equal. The rhizosphere-, root-, and, in the later part of work, litter-fungi, were handled uniformly but the number of isolation-replications varied, amounting to 12, 36, and 30 respectively.

Some further information on the extent of reliability of the methods demonstrated, especially in the case of soil fungi, were obtained from investigations on the influence of fungal communities on Fomes annosus carried out in the year 1965 in eight pine stands of a nearly healthy appearance. Four of those tree stands were chosen in the Forest Zielonka, where the Fomes problem is known to be of a low significance; four further tree stands in the Forest Laski, where Fomes annosus is considered as more dangerous. The general results are given in Table 3.

Table 3.--Summary effects of fungal communities, isolated from the soil environment of eight pine stands, on the development of Fomes annosus

Forest	Age of trees Section	Time of sampling	Roots	Rhizosphere	Soil	Litter
Zielonka	29	Spring	+ 42	- 302	- 110	+ 464
Zielonka	39c	Autumn	- 267	- 65	- 118	+ 442
Zielonka	53	Spring	+ 498	- 748	- 19	+2023
Zielonka	39f	Autumn	+1445	-1415	- 59	+ 142
Zielonka	31	Spring	+ 583	- 692	+ 1	+ 835
Zielonka	102c	Autumn	- 294	- 645	- 508	- 802
Zielonka	65	Spring	+ 739	- 510	+ 294	+ 663
Zielonka	88a	Autumn	+ 721	- 562	+ 31	+ 208
Laski	23	Spring	+ 700	- 432	-2059	- 445
Laski	17d	Autumn	+1058	- 429	- 383	+ 762
Laski	53	Spring	+ 196	- 926	-1036	- 17
Laski	29h	Autumn	- 95	-1910	- 545	+ 475
Laski	33	Spring	0	- 413	-1051	+ 992
Laski	63a	Autumn	+ 898	- 964	- 813	+1478
Laski	55	Spring	- 338	- 608	-1133	+1174
Laski	63f	Autumn	+ 874	- 344	-2344	+ 584

From the data given in Table 3 is evident that the tree stands in the Forest Laski may be more dangered by Fomes annosus than the tree stands in the Forest Zielonka, which is in accordance with the actual situation. This seems especially to be so when the summary effects of the soil fungi are examined. Similarly but not so strongly marked are the effects of the rhizosphere fungi. In contrast to these results, the summary effects on Fomes annosus of the communities of root- and litter-fungi were positive and of a more or less equal value. If one would like to add in algebraic manner the summary effects given in Table 3, the following results should be obtained:

Forest	Root-fungi	Rhizosphere-fungi	Soil-fungi	Litter-fungi
Zielonka	+3167	-4939	- 632	+4777
Laski	+3293	-6106	-9364	+5003

STUMPS OF PINUS SILVESTRIS AND THEIR COLONISATION BY FOMES ANNOSUS AND PENIOPHORA GIGANTEA

These investigations, carried out mainly by A. Przezborski, are not finished yet, but probably it would be of interest to present the currently available results.

Isolations of fungi were made from pine stumps in the Forests Zielonka and Laski. This was done at different time periods (1 month, 6 months, etc.)

after the stumps were established as a consequence of cutting trees. Fomes annosus was first isolated after 1 year of stump exposure; Peniophora gigantea after 1 month. More information was obtained from the results of isolations made from over 200 stumps in the Spring of 1967, although these stumps were established at different seasons (Spring and Autumn of the years 1965 and 1966). These results are presented in summary form in Table 4 and 5.

Table 4.--Absolute and relative numbers of fungal isolates obtained from pine stumps established in the Spring in comparison with those established in the Autumn

Forest	Spring			Autumn		
	Total	Peniophora gigantea	Fomes annosus	Total	Peniophora gigantea	Fomes annosus
Zielonka	1456	565	24	1860	133	26
	100%	39%	2%	100%	7%	1%
Laski	946	294	54	241	8	70
	100%	31%	6%	100%	3%	29%

Table 5.--Absolute and relative numbers of fungal isolates from the heartwood and from the sapwood of pine stumps

Forest	Isolates from heartwood			Isolates from sapwood		
	Total	Peniophora gigantea	Fomes annosus	Total	Peniophora gigantea	Fomes annosus
1	2	3	4	5	6	7
Zielonka	718	1	0	2598	697	50
	100%	0.1%	0%	100%	27%	2%
Laski	271	10	12	916	292	112
	100%	4%	4%	100%	32%	12%

From Tables 4 and 5 as well as from data not quoted here the following conclusions can be drawn: 1. Significantly more isolates of Peniophora gigantea were obtained from stumps established in the Spring than from the stumps established in the Autumn. 2. Significantly more isolates of Peniophora gigantea and of Fomes annosus were obtained from the sapwood than from the heartwood of the stumps. 3. Fomes annosus was isolated more frequently from Autumn stumps than from Spring stumps. 4. More isolates of Fomes annosus were obtained from the stumps of the Forest Laski than from the stumps of the Forest Zielonka, what is in conformity with the data given

in the former part of this paper (the pine stands of the Forest Laski are more affected by Fomes annosus than those of the Forest Zielonka). 5. The number of Peniophora isolates from the two forests involved were nearly equal. 6. Fomes annosus seems to colonise with preference stumps of younger trees; Peniophora shows a similar tendency though not so distinctly. 7. Fomes annosus and Peniophora gigantea were isolated more frequently from 4-5 cm. below the stump surface than from 1-2 cm.

Final Remarks

The Fomes annosus problem is undoubtedly of great complexity and therefore intensive studies on the ecology and biology of this fungus as well as on the characters of its host plants are necessary to solve this problem. Here was shown one of the possible ways--as we hope--of studying the ecology of the pathogen. More on this subject may be found in papers of Mańka, Blońska and Wnekowski (1960), Johnson and Mańka (1960), Mańka (1964), Gierezak (1967), Mańka (1967), etc. The second part of this paper deals partly with the role of the host in the infection process. Two further research projects of this type are now underway in our institution, one on the influence of the mode of treating of the tree-stumps (debarking, separating of larger roots, etc.) on the infection process, the other one executed together with the Institute of Dendrology in Kórnik, on genetic resistance in Pinus silvestris. Other Fomes annosus research-projects at the same institution are concerned with investigating the influence of different types of management (type of afforestation, type and time of thinning, etc.) on the resistance of tree stands.

On the basis of this paper control of Fomes annosus could be obtained by cutting the trees in the Spring, particularly when the tree stands are of a low age and when the forests coming into question are known to be susceptible ones.

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THE ACTUAL SITUATION OF RESEARCH ON THE DAMAGE CAUSED BY *FOMES ANNOSUS* IN FOREST STANDS IN ITALY

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The data recorded in this report have been obtained at the Forest Pathology Institute of Florence with the cooperation of Italian Forest Department (Corpo Forestale dello Stato). More particular research, which is here only partially reported, is conducted for the most interesting cases.

In Italy damage associated with growth of *Fomes annosus* on coniferous stands has been found on the following species:

Pinus nigra var. *calabrica* - on natural regeneration stands affected by sporadic dying on the highland of Sila (1000-1200 m. a.s.l.) Calabria. (1)

Abies alba - on young and adult plantations affected by group dying. On adult stands affected by heavy root rot and by heavy butt rot on the Apennines (600-1200 m. a.s.l.) and on the eastern Alps. (2 - 4 - 5 - 7 - 8 - 11)

Picea abies - (a) on adult plantations affected by heavy root rot and by butt rot on the northern Apennines (from 900 to 1200 m. a.s.l.). (5); (b) on adult plantations affected by heavy group dying on eastern Alps. (10 - 12); and (c) on natural stands affected by sporadic butt rot and group dying on eastern Alps. (11 - 13)

Larix decidua - on adult trees affected by root rot and by butt rot on the Apennines. (5)

Pinus pinea - on young and adult plantations affected by heavy group dying on the northern coasts of the Tirrenian and Adriatic Seas. (6 - 9)

Pinus pinaster - on young and adult stands affected by sporadic group dying of the northern coasts of the Tirrenian and Adriatic Seas. (6 - 9)

Cupressus sempervirens - on some plantations affected by sporadic root rot in central Italy. (3)

Pinus sylvestris - on young natural regeneration stands affected by sporadic group dying on eastern Alps. (11)

As we know three kinds of damage have been found in coniferous stands associated with the presence of Fomes annosus: group dying in young and adult plantations, and root rot and butt rot in adult stands. The adult trees affected by root rot may be windthrown. In Italy the heaviest damage affects the artificial stands of Abies alba, Picea abies, and Pinus pinea. Abies alba in the northern Apennines suffers the heaviest losses from root rot and butt rot in adult plantations of first rotation crops established on soil formerly cultivated. The adult plantations of Picea abies in first rotation crops in the northern Apennines suffer the same type of losses as Abies alba. In the eastern Alps the adult plantations of Picea abies (45-50 years old) in first rotation crops established, after the first world war, on soils formerly bearing agricultural crops or pasture, especially of calcareous nature, suffer in some cases from heavy group dying. In this case the course of the disease is very quick and even vigorous plants die in a period of 1-2 years. There too high soil pH (from 7 to 8) isn't suitable for Picea abies and therefore it may be that plants suffer from it and are more susceptible to disease. On the other hand the high soil pH may have a positive effect on the growth of Fomes annosus (Rishbeth, 1950, 1951a, 1951b, 1957; Wallis, 1960). One of the purposes of our research is to point out whether this process can be related with some meteorological influences.

Pinus pinea on the coasts of Tuscany suffers heavy losses from group dying only on the plantations established for the first time on soils formerly bearing agricultural crops or pasture. Where P. pinea is mixed with original wild vegetation the trees aren't damaged by Fomes annosus. In this case the fungus appears in most cases as one of the colonizers of pine stumps following the thinnings and clear-cut fellings.

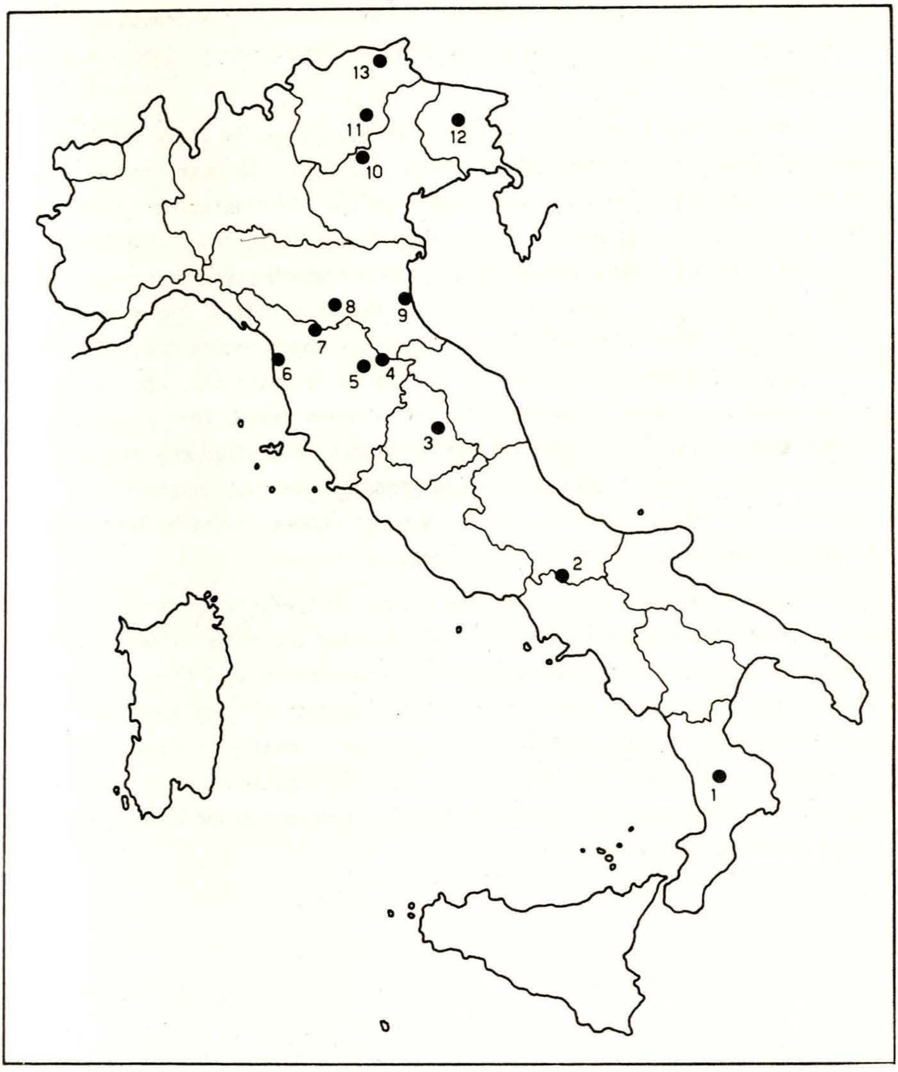

Figure 1.--Distribution of the attacks of Fomes annosus in conifer stands in Italy. (The numbers have been reported in the text).

Pinus pinea in the district of Ravenna is subjected to heavy attacks from Fomes annosus on roots. These attacks cause the death of young and old trees. They can be correlated with the damage which P. pinea suffers there from frost, and with high soil reaction (pH varies from 7.0 to 8.5). This

soil reaction, according to Rishbeth, is favourable to the growth of F. annosus. In the same soils Pinus pinaster mixed with P. pinea is rarely affected.

Generally the damages associated with F. annosus in Italy occur on plantations after thinnings and clear-cut fellings. We must consider that in many cases the first thinning is made on too old plantations; e.g. on 30-40-year-old plantations for Abies alba on the Apennines or for Picea abies on the Alps, and moreover on plantations which have remained too thick until that age; e.g. 3400-3500 plants per ha. in stands of Picea abies 35 years old. The regeneration of Pinus pinea sometimes is initially too thick; e.g. 5000-8000 plants per ha. in stands 12 years old. In many of these cases the stands appeared affected by heavy damage from F. annosus at the time of the first thinning; other stands in similar environments don't show any sign of attack by Fomes annosus. We must consider the action of all these factors on the subsequent damage caused by the growth of F. annosus as Rishbeth has written (1967).

The propagation of the infection occurs in the soil by the contacts among roots of infected stumps or diseased trees and roots of neighbouring healthy trees. We have clearly observed this fact in some Pinus pinea and Picea abies stands. In some young affected stands of Pinus pinea this fact isn't evident. Probably the propagation occurs directly through the soil. At present time laboratory research is also directed on the comparison of the several strains of F. annosus of Italian provenence and on the fungal flora found on conifer stumps.

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KERNFÄULEBEFALL ÄLTERER FICHTENBESTÄNDE (PICEA ABIES) AUF STANDORTEN IM SCHWÄBISCHEN JURA

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UNTERSUCHUNGSZIEL

In zahlreichen Untersuchungen ist die alte Erfahrung süddeutscher Forstleute bestätigt worden, wonach Fichtenbestände ganz verschieden stark von Kernfäule befallen werden, je nachdem, auf welchem Standort sie stocken (Übersicht bei Rohmeder, 1937 u. Werner, 1967). Die Ursachen für die beobachteten Korrelationen sind aber häufig noch unklar. Zum Teil mag das daran liegen, dass in den meisten bisherigen Erhebungen die Probestandorte nicht exakt genug charakterisiert wurden; ausserdem hat man sie gewöhnlich zu sehr heterogenen Gruppen als Befundeinheiten zusammengefasst (vgl. Kató, 1967a u. b; Zycha, 1964).

Im Rahmen eines grösseren, von der Deutschen Forschungsgemeinschaft unterstützten Rotfäule-Forschungsprogramms haben wir damit begonnen, die Standortsabhängigkeit des Kernfäulebefalls von Fichtenbeständen schärfer als bislang zu bestimmen. Wir benützen hierfür eine sehr differenzierte Gliederung der südwestdeutschen Waldstandorte. Dieser Bericht ist eine vorläufige Auswertung unserer ersten Aufnahmen im Wuchsgebiet "Schwäbische Alb".

Ausserdem sollen die Probebestände daraufhin überprüft werden, ob zwischen ihrem Ernährungszustand und dem Fäulebefall Zusammenhänge bestehen. Nach Braun (1958) kann eine physiologische Schwächung der Fichte die Infektion durch Fomes annosus fördern. Von landwirtschaftlichen Kulturpflanzen ist bekannt, dass die Versorgung mit Mineralstoffen die Infektionsbereitschaft gegenüber Bodenorganismen beeinflusst (Gerretsen, 1949). Laatsch wird auf dieser Konferenz darlegen, wie die Produktion fungizider Hemmstoffe in der Pflanze vom Ernährungszustand abhängt. Möglicherweise wirkt die Nährelementversorgung auf die ökologische Resistenz der Fichte gegen Kernfäuleerreger auch ein, indem sie den Zellwandbau und die Mykorrhizabildung beeinflusst (Björkman, 1967). Informationen zu diesem Fragenkomplex sind erwünscht, damit die Auswirkungen von Düngungsmassnahmen in Koniferenbeständen auf ihre Kernfäuledisposition beurteilt werden können (vgl. Seibt, 1964). Falls der Ernährungszustand für die Fäuleresistenz der Fichte bedeutungsvoll ist, eröffnen sich unter Umständen neue Wege für Prophylaxe und Bekämpfung (vgl. Rishbeth, 1967).

METHODIK

Als Grundlage unserer Erhebung dienen Standortskarten, die einheitlich nach den Prinzipien des südwestdeutschen forstlichen Standortserkundungsverfahrens erstellt sind (vgl. Schlenker, 1964). Alle Beobachtungen werden getrennt nach Standortseinheiten gasammelt. Diese sind sehr differenziert ausgeschieden, nach Geländemerkmalen genau definiert und in sich weitgehend homogen. In unserem Untersuchungsbereich entsprechen die meisten Standortseinheiten ganz bestimmten Bodenformen.

In zwei charakteristischen Teilgebieten des Schwäbischen Jura ("Mittlere Alb" und "Nördliche Ostalb") haben wir im Herbst 1967 insgesamt 61 rund 0,3 ha grosse, überwiegend reine Fichtenbestände im Alter von 58 bis 102 Jahren ausgewählt. Die beiden Untersuchungsareale liegen jeweils etwa 80 km S bzw. E von Stuttgart in 560-800 m Seehöhe. Ihre Zentren sind etwa 90 km Luftlinie voneinander entfernt. Die Jahresmitteltemperaturen schwanken zwischen 5,8 und 7,4° C, und die Jahresniederschläge variieren von 700 bis 950 mm. Die Probebestände repräsentieren 14 typische und grossflächig vorkommende Standortseinheiten und Bodenformen, auf denen die Fichte erfahrungsgemäss sehr unterschiedlich unter Kernfäule leidet. Unter den Kernfäuleerregern steht insbesondere auf den stark gefährdeten Standorten Fomes annosus an erster Stelle (Schönhar, pers. Mitteilung).

In jedem Bestand wird ein 0,1 ha grosser Probekreis optisch abgesteckt und gekluppt. Danach fällen wir 10 zufällig verteilte, herrschende Probebäume, die den Durchmesser des Grundflächenmittelstammes aufweisen. Sie sind äusserlich gesund, haben also keine erkennbaren Verletzungen und sind somit frei von Wundfäule. Aus dem Alter und der Schaftlänge dieser Bäume wird die mittlere DGZ_{100}-Bonität ermittelt, ausgedrückt in Vorratsfestmetern Derbholz je Jahr und Hektar (Hilfstafeln für die Forsteinrichtung Baden-Württemberg 1966). Wir betrachten den Anteil kernfauler Stämme unter den Probebäumen als grobes Schätzmass für den Kernfäulebefall im Bestand. Diese vorläufige Befallsschätzung soll später durch Anbohren oder zerstörungsfreie Prüfung stehender Bäume präzisiert werden. Den Ernährungszustand der Probebestände charackterisieren wir durch Analysen 1/2-jähriger Nadeln aus der Kronenspitze, getrennt nach gesunden und kranken Bäumen.

ERGEBNISSE UND DISKUSSION

Auch unsere grobe Stichprobentechnik hat deutliche und den Erfahrungen entsprechende Beziehungen zwischen den Standortseinheiten bzw. Bodenformen einerseits und dem Kernfäulebefall der Fichtenwälder andererseits aufgedeckt, wie die Übersichten 1 und 2 zeigen. Hierin sind die Standortseinheiten nach abnehmendem Befallsgrad angeordnet.

Am stärksten kernfaul sind die im Wuchs stockenden Fichtenaufforstungen auf Lehmrendzinen aus Malmdolomiten, auf Mergelrohböden und Mergelrendzinen aus Zementmergeln des Weissen Jura und auf Rendzinen aus obermiozänen Süsswasserkalken der mittleren Alb (Standortseinheiten "Dolomitverwitterungslehme", "Mergelrohböden" und "devastierte Tertiärkalkböden" der Übersicht 1). Alle diese Bodenformen zeichnen sich aus durch flache, sehr karbonatreiche, humusarme A_h-Horizonte, die von 1-4 cm mächtigen, schwach sauren Moderdecken überlagert sind. Die früher vorhandene, gut humose und vermutlich auch stärker entkarbonatisierte Krume ist während der landwirtschaftlichen Kultur verloren gegangen, sei es durch beschleunigte Mineralisation oder durch Abspülung am flachen Hang. In den alkalischen und an organischer Substanz verarmten Böden dürften die Voraussetzungen für eine Fomes-Infektion besonders günstig sein (Braun, 1958). Auch leiden diese Erstaufforstungen unter ausgeprägtem Stickstoffmangel; sie sind ausserdem verhältnismässig schlecht mit Mangan versorgt.

Übersicht 1.--Kernfäulebefall älterer Fichtenbestände auf charakteristischen Standortseinheiten der Mittleren Alb (n = 37; 1967)

Standortseinheit	Bodenform	n	Alter (Jahre)	Bonität (Vfm D/ ha/a)	alter Wald	Aufforstungen
					Anteil kernfauler Bäume (%)	
Dolomitverwitterungslehme	Lehmrendzinen	4	80- 91	4- 5/ 4	-	60-100/88
Mergelrohböden	Mergelsyroseme Mergelrendzinen	5	79-102	2- 7/ 5	-	18- 90/71
Devastierte Tertiärkalkböden	Moderrendzinen	3	80- 98	2- 5/ 4	-	77- 90/82
(Mässig) trockene Tertiärkalkböden	Mullrendzinen Lehmrendzinen	7	62- 95	6- 9/ 8	0-50/27	-
Flachgründige Kalkverwitterungslehme	Terrae fuscae	4	64- 83	8- 9/ 9	-	30- 90/56
Mittelgründige Kalkverwitterungslehme	Terrae fuscae	8	62- 82	10-12/11	10-37/21	50- 57/54
Schichtlehme	(Para) Braunerden/ Terra fusca-Relikt	3	62- 78	12-15/13	0-20/11	-
Feinlehme	(Para) Braunerden	3	66- 82	12-14/13	0-11/ 7	-

Übersicht 2.--Kernfäulebefall älterer Fichtenbestände auf charakteristischen Standortseinheiten der <u>Ostalb</u> (n = 24; 1967)

Standortseinheit	Bodenform	n	Alter	Bonität	alter Wald	Aufforstungen
			(Jahre)	(Vfm D/ ha/a)	Anteil kernfauler Bäume (%)	
Jura-Hänge	Lehmrendzinen	2	82-83	6- 8/ 7	20-64/42	-
Kalkverwitterungslehme	Terrae fuscae	4	78-87	8-10/ 9	9-20/16	50
Schichtlehme	(Para) Braunerden/ Terra fusca-Relikt	3	71-76	11-12/11	0	50-60/55
Schlufflehme	(Para) Braunerden	3	63-80	11-12/11	0-30/17	-
Oxalis-Myrtillus-Typ auf Feuersteinlehmen	Podsol-Braunerden Braunerde-Podsole	11	58-83	8-10/ 9	0-10/ 4	-
Myrtillus-Schreberi-Typ auf Feuersteinlehmen	Braunerde-Podsole	1	94	5	0	-

Fichtenbestände erster Generation nach Laubwald auf Mullund Lehmrendzinen aus Tertiärkalken (St. E. "trockene und mässig trockene Tertiärkalkböden" der Übersicht 1) sowie auf Lehmrendzinen an Jura-Steilhängen (Übersicht 2) sind ebenfalls stark von Kernfäule bedroht. Die Befallsgrade liegen aber niedriger als für die erstgenannte Gruppe von Standortseinheiten. Die A_h-Horizonte dieser Böden reagieren zwar in der Regel noch basisch, doch sind die Karbonatanteile geringer und die Humusgehalte höher als auf jenen ehemaligen Acker- und Weideflächen. Vermutlich ist unter diesen Bedingungen die Aktivität der Fomes-Antagonisten nicht mehr so stark beeinträchtigt (vgl. Low und Gladman, 1960; Greig, 1962).

Mässig wüchsige Fichtenwälder auf flach- und mittelgründigen Terrae fuscae aus verschiedenen Malmkalken (St. E. "Kalkverwitterungslehme") erwiesen sich als verhältnismässig gesund, soweit es sich um alte Waldböden handelt (vgl. Moreau und Schaeffer). Sie wurden zumindest in den vergangenen 2 bis 3 Jahrhunderten nicht landwirtschaftlich genutzt und trugen als Vorbestand meistens Laubwälder. Ihre A_h- und B_v-Horizonte sind abgesehen von einzelnen Gesteinstrümmern frei von Karbonaten; die pH-Werte (nKCl) variieren zwischen 4 und 6. Trotzdem ist die relativ hohe Stabilität dieser Bestände erstaunlich, denn in 30 bis 50 cm Tiefe erreichen die Fichtenwurzeln hier überall karbonatreiche und humusarme B_vC-Übergangshorizonte, wo sie einem starken Fomes-Angriff ausgesetzt sein sollten (Braun, 1958). In Fichtenaufforstungen auf Terrae fuscae ist die Kernfäule im Gegensatz zu den Beständen nach Laubwald jedoch weit verbreitet. Wir vermögen indessen noch nicht anzugeben, wie die frühere Beackerung und Beweidung auf diesen Standorten die Bodeneigenschaften und den Ernährungszustand der neubegründeten Wälder beeinflusst haben.

Auf (Para) Braunerden aus (meist äolischen) Deckschichten wechselnder Mächtigkeit über Terra fusca-Relikten (St. E. "Schichtlehme", "Feinlehme" u. "Schlufflehme") bleiben die sehr wüchsigen, mit Makro- und Mikronährelementen vorzüglich versorgten Fichten (in der Regel 1. Generation nach Laubwald) bis ins hohe Alter ziemlich gesund. Ihre Standorte zeichnen sich aus durch kräftig humose Mull-A_h-Horizonte, und die pH-Werte liegen zumindest im Hauptwurzelraum im sauren Bereich.-Lediglich die Aufforstungen auf Schichtlehmen sind wiederum erheblich kernfäulegefährdet. Vermutlich gilt diese Feststellung auch für Aufforstungsbestände auf Feinlehm- und Schlufflehm-Standorten.

Die im Rahmen unserer Erhebung am schwächsten von der Kernfäule betroffenen Fichtenbestände stocken auf Podsol-Braunerden und Braunerde-Podsolen der Ostalb (St. E. "Oxalis-Myrtillus-Typ" und Myrtillus-Schreberi-Typ" aus Feuersteinlehmen-vgl. Übersicht 2). Diese haben sich aus mächtigen, alten, feuersteinführenden Verwittergunsdecken über Jurakalken entwickelt, tragen Rohhumusauflagen und reagieren bis weit in den Unterboden hinein stark sauer. Bei den überprüften Beständen handelt es sich durchweg um die 1. Fichtengeneration nach artenarmen Buchenwäldern. Sie leiden unter Stickstoffmangel, der jedoch im Unterschied zu den stark kernfaulen Fichtenaufforstungen auf devastierten Rendzinen der mittleren Alb mit einer normalen Manganernährung kombiniert ist.

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CURRENT RESEARCH IN THE CONTROL OF *FOMES ANNOSUS*

WITH *SCYTALIDIUM* SP., AN IMMUNIZING COMMENSAL

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The investigation of immunizing commensality for the control of wood pathogens in live trees is just beginning in Oregon. It stems, in part, from the encouraging results obtained in utility poles with a local *Scytalidium* sp. for the control of a major heartwood destroyer in Douglas-fir utility poles, *Poria carbonica* Overh.

PREVIOUS WORK

During a survey of the incidence of wood destroying fungi in the untreated wood of Douglas-fir utility poles in service in Western Oregon (Ricard, 1966), a *Scytalidium* sp., coded FY, was found to occur often singly in a number of poles free from visible decay. The relationship of this fungus with the wood and with *P. carbonica* was investigated (Ricard and Bollen, 1968). It indicated that FY could develop in Douglas-fir without damage to the important functional properties of the wood thereby exhibiting the characteristics of a commensal in that substrate. Yet the FY showed strong antagonism toward *P. carbonica* *in virtro*, when cross plated on malt extract agar using agar or wood blocks as inoculants. This

antagonism is due at least in part to modification of the substrate resulting from the growth of FY, including release of an antibiotic substance. Wood permeated with FY mycelium remains resistant to attack by _P. carbonica_ even after the FY hyphae have been killed by heating with flowing steam.

Field experiments (Ricard and Bollen, In preparation) indicated that FY could be introduced artificially in Douglas-fir poles infected with _P. carbonica_ at the incipient decay stage and become established in portions of the poles previously occupied by the wood pathogen. No live _P. carbonica_ was recovered using the field procedure described earlier (Ricard and Mothershead, 1966) from those areas of the poles where FY mycelium had become established.

RELATIONSHIP OF _SCYTALIDIUM_ SP. "FY" AND _F. ANNOSUS IN VITRO_

Earlier work by Klingstrom and Beyer (1965) showed that _Scytalidium album_ and _aurantiacum_ were effective against _F. annosus_. These findings stimulated the evaluation of FY against cultures of _F. annosus_ recently isolated from western hemlock by Laird (1968). He reports that "the conventional cross plating technique" was used, which involves the placement of agar cubes containing _Fomes annosus_ and FY on opposite sides of a petri dish containing 2.5% malt agar."

An additional technique, which tests the antagonistic ability even more severely, was also employed. In this method a cube of agar containing _F. annosus_ was plated in the center of the petri dish containing 2.5% malt agar. Ten days later an agar cube of FY was placed on the mycelial mat of _F. annosus_.

The test plates were then examined for antagonistic activity six weeks after the initial inoculant was placed on the agar. An attempt to isolate _F. annosus_ from the test plates was then made using the selective agar for _F. annosus_ developed by Kuhlman and Hendrix (1962). In the cross inoculation plates the advancing mycelium of the two fungi met on the FY side of the plate center indicating that _F. annosus_ growth rate exceeds that of FY on malt agar. When the two colonies first meet a zone of inhibition forms approximately 1 mm. wide. It persists only until penetrated by the yellow water soluble pigment of FY. This occurs generally from two to three days after the colonies have met. Once the zone is penetrated by the yellow pigment, FY has no trouble following and in a two to three week period of time both the pigment and the FY mycelium have penetrated the zone formerly occupied by _F. annosus_.

When the FY agar cubes are placed on plates already colonized by F. annosus, pigment production, diffusion and mycelial invasion again occur. The rate of movement is slow at first but picks up momentum as the area colonized by FY increases and within one month after inoculation of the plates with FY agar cubes the plate is completely colonized by FY.

Regardless of the method used, six weeks after introduction of the FY inoculant, chlamydospores appear in varying concentrations. In all of the plates the pigment has completely diffused through the agar. Attempts to recover F. annosus from the mixed colonies on the Kuhlman-Hendrix medium failed. This suggests that F. annosus is not only overgrown but destroyed directly by FY.

It should be emphasized that these tests were conducted using a strong pigment producing strain and that the results would differ if strains producing less pigment were used, as pigment and antibiotic releases appear to be associated.

INOCULATION TECHNIQUE FOR FIELD TRIALS

Personal communications by Hüppel and Klingstrom (1967) indicated that Scytalidium sp. could be introduced artificially in live trees, so that field evaluation of FY against F. annosus appeared to be warranted. However the rapid inoculation technique used for the inoculation of Douglas-fir poles was found to be unsuitable for live tree inoculation.

A modification of the FY dart technique, the FY bullet, was developed and appears to be promising for live tree inoculation. The inoculant is a section of birch doweling permeated with FY, about 3 cm. long and 0.6 cm. in diameter. One end is forced into a cast zinc alloy arrow tip weighing about 3.5 grams. Epoxy resin is used to bind tip and doweling together. The assembled inoculant is placed in a 30-06 caliber shell and fired, with a low powder charge to avoid tumbling from a standard rifle of that caliber. The FY permeated doweling penetrates through the bark into the wood to a distance averaging 5 cm. Penetration depth can be adjusted with powder change and hardness of the wood stock used for the bullet.

FY bullets fired into poles were removed aseptically and plated on malt extract agar. Upon incubation, typical FY colonies developed suggesting that the viability of the fungus was not affected strongly by the inoculation procedure.

APPLICATION TO CONTROL OF F. ANNOSUS

As indicated by Rishbeth (1967), F. annosus survival is influenced by the occurrence of competitors. Scytalidium sp. is one such organism with the particular advantage of a commensal-type relationship with the hostwood. While Douglas-fir appears to be the Scytalidium host which has received more attention, it is known that Scytalidium can develop in a number of tree species. Klingstrom and Beyer (1965) have isolated Scytalidium sp. from Scots pine and Norway spruce. Pesante (1965) made the original isolation of Scytalidium lignicola from plane. FY was observed to grow in specimens of several hard and softwoods obtained through the courtesy of Mr. C. Jacquiot's group at the Centre Technique du Bois in Paris. Pinus sylvestris appeared to support particularly rapid growth of FY.

A possible utilization of FY could be its preventive introduction in the wood of live trees, so that it impregnates the substrate with antibiotic as the mycelium develops, immunizing the host against subsequent invasion by F. annosus or other wood destroying basidiomycetes sensitive to FY metabolites.

CURRENT PROBLEMS

Major questions are raised by such preventive application. They include the stability of the antibiotic in the wood over the years and perhaps most important the constancy of antibiotic release by Scytalidium sp. Laboratory tests completed so far do not preclude substantial stability of the antibiotic since it is not soluble in cold water, remains active after storage at room temperature in wood for six months and is not affected by moist heat near 100° C. Isolation and chemical identification of the antibiotic is needed for a more accurate assessment of its potential.

In the area of constancy in antibiotic release, the observations made in Oregon appear to be consistent with those made by the Swedish workers, the various Scytalidium sp. are not identical in their antagonistic properties. In FY the antibiotic release varies with the environment and depends particularly on the occurrence of certain competing basidiomycetes. When FY develops in such competing system, simultaneous release of antibiotic and yellow, water-soluble pigment takes place. However when FY grows in vivo or in vitro in a competitor-free environment, it soon stops the release of antibiotic in a fashion typical of an anabolic mechanism controlled by inducible enzymes (Stanier et al., 1963). This characteristic

implies that some provision will have to be made for the proper stimulation of the immunizing commensal. Recent developments in microbial genetics and physiology make two approaches particularly inviting to secure constancy in the synthesis of antibiotic by FY: (1) development of an FY mutant free from the suppressor repressing the activities of the regulatory operon governing the synthesis of the enzymes necessary for the formation of the antibiotic; (2, identification of the metabolite released by F. annosus, P. carbonica and other certain basidiomycetes which triggers the release of antibiotic by FY, presumably by inhibition of the suppressor.

While solutions are developed for some of these problems, Scytalidium sp. should find application in the curative treatment of trees and poles at the incipient decay stage from infections with basidiomycetes sensitive to FY metabolites, particularly F. annosus, F. subroseus, Poria weirii, and Poria carbonica.

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THE POSSIBILITY OF STUMP INOCULATION FOR CONIFERS OTHER THAN PINES

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INTRODUCTION

Experiments in Great Britain have shown that inoculating stumps of pines, *Pinus sylvestris* and *P. nigra* var. *calabrica*, with *Peniophora gigantea* gives excellent protection against their infection by air-borne spores of *Fomes annosus*. *P. gigantea* tends to check advance of the parasite in any roots infected at the time of felling and also replaces it to some extent (Rishbeth, 1963). This method is increasingly used in British pine plantations, the present area so treated being about 70,000 acres (Rishbeth, 1967). The Forestry Commission is interested in the possibility of devising a suitable inoculation method for stumps of Sitka spruce, *Picea sitchensis*, extensively planted in the north and west of Britain, to replace the present treatment with sodium nitrite. Inoculation methods suitable for stumps of other conifers might also be useful. Although *P. gigantea* may grow on stumps of such conifers, there is some evidence that it is less effective for controlling *F. annosus* than with pines (Rishbeth, 1963). The progress made in this area of research so far is briefly outlined here.

INOCULATION OF SPRUCE STUMPS

It was difficult to make a realistic selection of fungi for field experiments. Preliminary surveys made by the Research Branch of the Forestry Commission indicated that fructifications of polypores, in particular, were scarce on Sitka spruce stumps in the relatively young British forests, but a small collection of stump fungi was nonetheless obtained. The detailed investigation by Käärik and Rennerfelt (1957) of fungi present on stumps of Norway spruce, Picea abies, suggested several species which might be tried with this conifer but which would not necessarily be useful with Sitka spruce. Selected cultures of fungi already known to be present in Britain were obtained through the courtesy of Dr. Käärik. Other fungi were obtained from the Forest Products Research Laboratory, Princes Risborough, and from suitable material in East Anglian plantations, where the subsequent forest trials were carried out. Since there were not enough spruce stumps available at the time in this area to test the whole collection of twenty-six fungi, attempts were made to screen them in the laboratory. The main criteria used were ability to grow in lengths of fresh spruce wood and to compete with F. annosus in them, but the tests were not altogether satisfactory owing to frequent contamination of the lengths with Trichoderma. The species tested were as follows, the figure in brackets referring to the number of isolates, if more than one: Corticium laeve, Peniophora gigantea, P. pithya, P. sanguinea, Stereum sanguinolentum, Trechispora brinkmanni (2), Irpex sp., Tremellodon gelatinosum, Polyporus adustus, P. amorphus, P. benzoinus, P. borealis (2), P. stipticus, Polystictus abietinus, P. versicolor, P. sp., Fomes pinicola (2), Lenzites sepiaria (2), Pleurotus mitis, P. porrigens, Pholiota squarrosa, Tubaria furfuracea, Flammula sp., Cortinarius sp., Hypholoma fasciculare and Trichoderma viride (3).

With the selected fungi, inoculation experiments were set up on freshly cut stumps of healthy spruce, all of which received a standard dosage of about 1×10^3 viable basidiospores of F. annosus. Inoculum of twelve potential competitors was prepared in the form of a suspension of asexual spores, basidiospores, or fragmented mycelium. For any given fungus the inoculum was standardized, but no attempt was made to apply the same dosage for all of them: dosages varied in fact from 5×10^3 to 1×10^6 viable units per stump. For each competitor, ten Sitka spruce stumps were inoculated; a further set of ten received F. annosus only. A parallel experiment was set up with Norway spruce, for which however shortage of

stumps permitted the testing of only nine species. From each treatment two samples, obtained by sawing off a disc about 4 cm. thick from the stump surface, were collected after 6 months, but growth of fungi was so limited that the remaining stumps were not sampled until 16 months had elapsed.

With Sitka spruce, the mean area of sapwood in the stump section occupied by F. annosus after the latter period varied from 4-19% (mean 10%) for the control (without competitor) and for stumps also inoculated with Trechispora brinkmanni, Polyporus adustus, P. benzoinus, P. borealis, Polystictus sp., Lenzites sepiaria, Pleurotus mitis, P. porrigens, Hypholoma fasciculare or Trichoderma viride. Variation within treatments was considerable since many stumps were not colonized by wood-rotting fungi even after this period: a far greater number of replicates would be required to detect significant differences between treatments. By contrast stumps inoculated with Stereum sanguinolentum (dosage 9×10^4 basidiospores) or Peniophora gigantea (dosage 1×10^6 oidia) contained no F. annosus even though the area of sapwood occupied by these fungi was only 24% and 17% respectively. The heartwood had not been colonized in any treatment. Four stumps inoculated with S. sanguinolentum and four with P. gigantea were excavated and lateral roots removed for examination: F. annosus was absent from the two sets of sixteen roots so obtained. However, of fifteen roots taken from four stumps inoculated with Pleurotus porrigens, seven contained F. annosus. Thus in this experiment the majority of fungi failed to control the parasite. S. sanguinolentum and P. gigantea were effective in this respect but disappointing in that they grew so slowly. It may be noted that the dosage of P. gigantea applied was about a hundred times that required to give good control in pine stumps.

Stumps of Norway spruce were sampled similarly, and the mean area of sapwood in the stump section occupied by F. annosus was found to be higher than with Sitka spruce, ranging from 20-40% for the control and stumps inoculated with Polystictus sp., Pleurotus porrigens or Hypholoma fasciculare. Amounts of F. annosus infection (7-12%) rather less than the control were present in stumps inoculated with Trechispora brinkmanni, Polyporus benzoinus or Pleurotus mitis; however in these series natural infection by Polyporus adustus had occurred, which might account for the partial suppression of F. annosus. No infection with the latter was observed in stumps inoculated with Polyporus adustus or Peniophora gigantea, and only a minute infection occurred in one stump inoculated with Stereum sanguinolentum. The mean percentage area of stump section occupied by these

fungi was 77, 72 and 23 respectively, so that S. sanguinolentum had grown no better than in Sitka spruce stumps. With Norway spruce P. adustus therefore appears to be an effective competitor, in addition to the two fungi performing best with Sitka spruce. A more extensively replicated experiment with the former conifer might well show that some of the other fungi exert partial control over F. annosus. Polyporus borealis, Lenzites sepiaria and Trichoderma viride were not tested.

Several conclusions may be drawn from this experiment. Firstly, a number of fungi which appeared promising from laboratory tests were ineffective under forest conditions and no fungus proved to be a better competitor than it was in the laboratory. This result is perhaps hardly surprising, and reduces the chance that any potentially valuable competitor in the present collection was overlooked. Secondly, stumps of Sitka spruce differed in two main respects from those of Norway spruce: they were colonized very slowly and by relatively few wood-rotting fungi and, partly no doubt because of this, F. annosus was almost as abundant in the sapwood as any competitor. With Norway spruce sampled after an equivalent period, more advanced stages of fungal succession were encountered and F. annosus was generally much less abundant than its competitors. It is perhaps worth stressing that in pine stumps inoculated jointly with comparable dosages of F. annosus and P. gigantea, the former at first becomes established and is replaced only later: the results obtained by stump sampling thus depend on its timing in relation to rates of fungal growth.

Of the two fungi which competed well with F. annosus in Sitka spruce stumps, Stereum sanguinolentum might well be ruled out for practical purposes. Not only is it unlikely, from the evidence obtained with pine wood (Rishbeth, 1950), to replace F. annosus in any established infections, but it is so widely implicated as a wound-pathogen (Pawsey and Gladman, 1965) that to increase its population in forests through stump inoculation seems undesirable. These objections do not apply to Peniophora gigantea, but its slow growth in stumps of Sitka spruce is a disadvantage. Moreover, in a similar experiment set up by the Forestry Commission Research Branch in Wales, P. gigantea did not prevent, although it greatly reduced, infection of F. annosus in freshly cut Sitka spruce stumps. When inoculation with P. gigantea was delayed for 8 hours or when stumps immediately inoculated with this fungus were damaged after 2 weeks and then re-inoculated with F. annosus, the latter was found in a greater proportion of stumps. These results, which differ markedly from those obtained with Scots pine, probably

reflect the slow growth of P. gigantea in Sitka spruce stumps already mentioned. The failure of P. gigantea to control F. annosus in stumps of European larch, Larix decidua, within about a year of felling has been recorded earlier (Rishbeth, 1963). On balance, therefore, simple inoculation with P. gigantea cannot be considered safe enough for general use.

In this situation two possibilities were envisaged. Further search for competitors might reveal fungi more effective than those found hitherto, and therefore methods for collecting such fungi were discussed with the Forestry Commission Research Branch and put into operation. However, although the collection described earlier constitutes only a small proportion of the fungi which might be tested, it cannot be assumed that species really suitable for use with Sitka spruce exist in Britain. This consideration leads to another possibility, that more reliable ways might be found of establishing P. gigantea in stumps of Sitka spruce and perhaps those of other conifers. Although this seems an unenterprising solution, in view of the great variety of stump fungi occurring naturally, such a procedure would have the great practical advantage that only one type of inoculum would be required for stands of several different conifer species, or indeed for stands comprising a mixture of conifers. And whereas a method of producing P. gigantea inoculum is already in operation, it might prove more difficult to produce suitable inocula for other fungi, especially if these formed no asexual spores in culture.

FURTHER EXPERIMENTS WITH PENIOPHORA GIGANTEA

The starting point for this work was an observation in an earlier experiment with European larch. After 11 months, P. gigantea had occupied only 20% of the area of cross-section in stumps inoculated with the fungus and yet, as a result of natural infection, it had occupied 40% in uninoculated stumps treated with 20% ammonium sulphamate. The marked tendency for pine stumps treated with this compound to become colonized by P. gigantea has been noted before (Rishbeth, 1959). It was thought that a discovery by Punter (1963) might also be exploited. When freshly cut pine stumps were treated with 20% sodium thiosulphate and then inoculated with both F. annosus and P. gigantea, the former was suppressed within 3 months and the latter grew as well as in controls treated with water. This effect

is of little practical importance with pines, since F. annosus is later adequately suppressed by P. gigantea in the absence of thiosulphate, but it might be more important with other conifers.

An experiment was set up to determine whether addition of various solutions to freshly cut wood discs influenced their colonization by P. gigantea. No attempt was made to study interaction with F. annosus. The conifers selected were Sitka and Norway spruce, European larch and Douglas fir, Pseudotsuga menziesii. The main interest centred around the effects of ammonium sulphate, ammonium sulphamate, sodium thiosulphate and ammonium thiosulphate. Preliminary trials showed that solutions of 5 and 10% strength were most appropriate for the first three compounds and solutions of 2.5 and 5% strength for the last one. With all conifers better colonization occurred after treatment with some of the solutions than in water-treated controls. The most effective were 5% ammonium sulphate or sulphamate for discs of both types of spruce, these same treatments together with 2.5% ammonium thiosulphate for those of Douglas fir, and 5% ammonium sulphate for those of European larch.

In view of the possibility that correlation between performance in the laboratory and that in the forest might be poor, it was decided to repeat the full range of treatments in subsequent experiments with freshly cut stumps of the four conifers. Nine series of ten stumps each were first inoculated with F. annosus and P. gigantea, the dosages of which were standardized for any given conifer. After 2 hours they were given the appropriate chemical treatment (eight series) or water only (control series). A further set of ten stumps was inoculated with F. annosus only. At intervals ranging from 17-22 months, stumps were sampled as before. The results are given in Table 1, those for Norway spruce being very tentative because shortage of stumps limited the range of treatments and because subsequent disturbance in the area reduced the number of replicates available for sampling.

It may first be noted that only with Douglas fir was a natural inoculum of P. gigantea critically scarce: its abundance in stumps inoculated with F. annosus alone was a minute fraction of that in stumps also inoculated with P. gigantea, whereas with the other conifers this fraction was about a half. Witn Sitka spruce it is not clear why growth of both fungi was so much slower than in the previous experiment. The area occupied by P. gigantea in stumps treated with water was in fact so small (3%) that the increase resulting from treatment with 5% ammonium sulphamate (35%) is all the more striking. In view of the very small amount of F. annosus in stumps

Table 1.--The effect of various chemical treatments on growth of P. gigantea and F. annosus in conifer stumps, and on interaction between them

	Conifer and period after felling (months):		Sitka spruce (24)		Norway spruce (17)		Douglas fir (17)		European larch (22)	
			The mean percentage area of sapwood in the stump section occupied by the fungi in:							
Inoculation	Chemical treatment	Concn. (%)	Pg[+]	Fa	Pg	Fa*	Pg[+]	Fa	Pg[+]	Fa*
Fa + Pg	Ammonium	10	16	2	31	4	65(0.2)	4	10	2
Fa + Pg	sulphate	5	3	0	-	-	73(0.1)	0	21	17
Fa + Pg	Ammonium	10	12	0	26	3	95(0.9)	0	36(0.2)	2
Fa + Pg	sulphamate	5	35(0.2)	0	-	-	100(1.3)	0	32(0.3)	8
Fa + Pg	Sodium	10	1	0.2	48	3	49	0	62(1.3)	4
Fa + Pg	thiosulphate	5	6	0	-	-	27	0	40)0.7)	2
Fa + Pg	Ammonium	5	2	0	-	-	16	0	36(0.6)	6
Fa + Pg	thiosulphate	2.5	0.5	0	-	-	37	0.5	15(0.1)	7
Fa + Pg	Water		3	0	32	5	20	0	34(0.3)	13
Fa only	Water		1.5	2	14	12	0.3	15	17	15

[+]Figures in brackets refer to the mean abundance of P. gigantea sporophores scored by rating 0-3.

*Variable but often extensive amounts of heart-rot were present at the time of felling. Figures refer to areas occupied by F. annosus since felling, both from this source and from spore inoculum.

inoculated with this fungus only, no firm conclusions can be drawn about the effect of the various other treatments on its occurrence. In general, however, inoculation with P. gigantea seems to have controlled F. annosus, as in the experiment described earlier.

Similarly, little can be deduced from the limited data obtained from Norway spruce. Growth of the two fungi was more extensive than in Sitka spruce, again as in the previous experiment. Failure of P. gigantea to control F. annosus altogether, as it did previously, was associated with the presence of the latter in heart-rotted tissue prior to felling. Of the three chemical treatments given, 10% sodium thiosulphate was the only one apparently favouring increased growth of P. gigantea, but its effect was very erratic. Further experiments with this conifer are in progress.

The results obtained with Douglas fir are perhaps the most striking. The area of cross-section occupied by P. gigantea was greatly increased by treatment with ammonium sulphamate or sulphate, the former being more effective. Sporophores of the fungus were often present on stumps so treated and there was a reasonably good correlation between their abundance and the extent of growth in the wood, both with this conifer and with European larch. In an earlier experiment with Douglas fir (Rishbeth, 1963) stumps inoculated with both F. annosus and P. gigantea were found after 5 months to have 28% of the cross-section occupied by the former species and 20% by the latter. In the current experiment, stumps to which only water was added and for which the respective dosages were similar still contained only 20% of P. gigantea after 17 months but had no F. annosus. As with Sitka spruce stumps, in which incidentally F. annosus was detected at a preliminary sampling after 8 months, it appears that even where growth of P. gigantea is relatively limited, this fungus can eventually replace the former one. This does not happen after all chemical treatments; however, 10% ammonium sulphate tended to favour survival of F. annosus even though it led to increased growth of P. gigantea. The reason for this effect, also seen with Sitka spruce, is not known.

With European larch application of ammonium sulphamate did not increase growth of P. gigantea, as expected from the observation quoted earlier, the only treatment which did so being 10% sodium thiosulphate. The mean area occupied by F. annosus in the various treatments was affected somewhat erratically by the incidence of heart-rot. An attempt was made to distinguish between infections of the sapwood arising from this source, which had extended outwards for distances up to 2 cm. in the period since felling, and those derived from spore inoculum. Two treatments, 10% sodium thiosulphate and 10% ammonium sulphate, apparently resulted in complete

elimination of the latter type of infection by P. gigantea. In a few instances this fungus was replacing F. annosus in the heart-rotted area. In stumps inoculated with P. gigantea and receiving only water there was little control of F. annosus derived from spore inoculum, by contrast with Douglas fir.

DISCUSSION

There was rather poor agreement between these results, as affecting establishment of P. gigantea, and those obtained in the laboratory experiment on wood discs. With Norway spruce the results are very few but resemble those obtained with European larch. With the three other conifers, one or more treatments fulfilled the major aim of controlling any F. annosus which entered through the cut surface. Despite the tendency for P. gigantea to control such infection in the absence of any chemical treatment, given time, the result obtained with European larch confirms the earlier conclusion that inoculation alone is unreliable.

In considering the other important aim of stump treatment, that of limiting growth of F. annosus from stump tissues already infected at the time of felling, attention is naturally directed to treatments favouring rapid establishment of P. gigantea. For Sitka spruce and Douglas fir treatment with 5% ammonium sulphamate, and for European larch treatment with 10% sodium thiosulphate appear in this respect the most promising of those tested. In stumps of heart-rotted trees the advantage for colonizing fresh wood which F. annosus has over any competitor introduced at the surface increases with distance below this, hence the faster the competitor grows the better. Under the conditions of these experiments wood-rotting fungi grew more slowly in stumps of the four conifers than had been observed before in pines, and therefore the outcome of competition between them was correspondingly delayed. It is by no means clear to what extent replacement of F. annosus in tissues already infected at the time of felling can be expected, though results with European larch are mildly encouraging.

It might be argued that with Sitka spruce and Douglas fir treatment with 5% ammonium sulphamate alone would give the desired effect. When sufficient natural inoculum of P. gigantea or other effective competitors exists, this might well be so, but the experiment with Douglas fir shows again that such inoculum is not always available (Rishbeth, 1963). Experiments by Punter (1963) on pine stumps demonstrate that treatment with 20% ammonium sulphamate alone is not sufficient to prevent entry of

F. annosus. Apart from these considerations it seems likely that in many cases a sufficiently rapid build-up of competitors in stumps will only be achieved by deliberate inoculation.

For large-scale application of such methods in the forest it needs to be shown that they are superior in their effects to existing ones, which still has to be demonstrated, and that they are convenient and not too costly. Fortunately the viability of P. gigantea oidia is unaffected by 5% ammonium sulphamate or 10% sodium thiosulphate over a period of 8 hours, although with the latter it declines over a period of 24 hours. Inoculation may thus be combined with chemical treatment. Therefore although treatments would require a little more preparation and would be slightly more expensive, they do not seem impracticable.

The question of whether P. gigantea, though convenient, is the best fungus to use is still open. It grows only slowly into stump roots of Douglas fir, for example, even when present extensively in the body of the stump: this may reflect its basic unsuitability for use with this conifer. Conceivably strains of P. gigantea will be found which grow much more freely in stump tissues of conifers other than pine, but so far as is known there is no evidence for this. Even if better competitors are discovered it may prove desirable to enhance their growth by chemical means. Clearly there is much to explore in this field of applied ecology. Interaction between fungi in stumps is complex, and we need to know far more about the growth requirements and mechanisms of competition of important species such as P. gigantea. The effects of chemical treatments further complicate the picture and it is important to discover to what extent reproducible results can be obtained; ultimately a more fundamental approach to the problem is desirable.

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STUMP AND SOIL TEMPERATURES IN A SLASH PINE STAND AND THEIR RELATION TO COLONIZATION BY *FOMES ANNOSUS*

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Within the past two decades, intensive management of large acreages of slash (*Pinus elliottii* var. *elliottii* Engelm.) and loblolly (*P. taeda* L.) pines has become an important means of pulpwood production in the southeastern United States. Concomitant with this intensified plantation management, timber losses associated with *Fomes annosus* (Fr.) Karst. have increased dangerously. If economic production of pulpwood in this area is to continue, more must be known about the fungus and the factors which affect its growth.

Application of powdered borax to freshly cut stump surfaces (Driver, 1963) is commonly used to prevent stump infection by *F. annosus*. More recently it has been found that seasonal thinning may also be useful in reducing losses from this disease (Ross, 1967; Ross and Driver, 1966). Driver and Ginns (1964) postulated that high temperatures may limit the ability of *F. annosus* to colonize freshly cut stumps during the summer. Gooding, Hodges, and Ross (1966) found that actively growing mycelium of *F. annosus* in wood was killed in the laboratory after a 2-hour exposure to 40° C.; preliminary studies indicated that this temperature is reached in

stump surfaces under field conditions. This paper reports the results of more detailed studies on stump and soil temperatures and their relationship to stump infection by F. annosus.

METHODS

The study was established in a 15-year-old slash pine plantation, spaced approximately 6 x 8 feet, located near Bainbridge, Georgia. Twelve plots were established each month for 12 consecutive months. The stand in which the 144 plots were established was thinned operationally from an original stocking of 160 sq. ft. of basal area to 80 sq. ft. Each month, stump surfaces in six plots were inoculated with a conidial suspension of F. annosus immediately after felling and six plots were not inoculated, except for natural air spora of this fungus. Half the plots of each treatment were preserved to study the spread of F. annosus to the residual trees. Two to three months after plot installation, 25 stumps in each of the remaining plots were sampled, quartered, and isolations were made from each face of the quarters to determine the degree of colonization of F. annosus.

At the time of plot installation, in each series of plots, temperature-sensing probes were placed in eight stump tops approximately 1/4 inch beneath the surface, in the air adjacent to and level with four stump tops, and in the soil near two stumps 6 inches deep. Temperatures were recorded continuously throughout the study. Every 2 months the temperature recorders were moved to a new location within the study area.

RESULTS

The percentage of stumps colonized by F. annosus from February 1965 to January 1966 in this study has been previously reported (Ross and Driver, 1966), but for comparative purposes the results are graphically presented here (Figure 1). These data show no natural infection from April through August and the same general trend in the inoculated stumps, with the exception of slightly greater numbers of stumps being colonized. This study also shows that stump and air temperatures reached the thermal inactivation point (40° C.) for actively growing mycelium during the first week of April and continued to do so for several consecutive days until mid-September (Figure 2). The highest stump temperature during this study was 47° C. Soil temperatures during this period ranged from a high of 35° C. to a low of 13° C. (Figure 2).

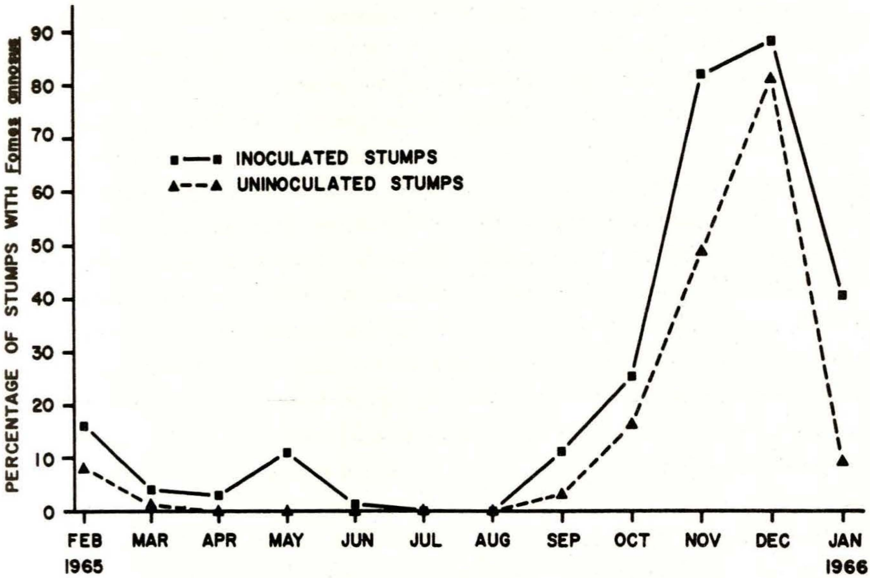

Figure 1.--Percentage of stumps colonized by Fomes annosus in seasonal thinning study at Bainbridge, Georgia

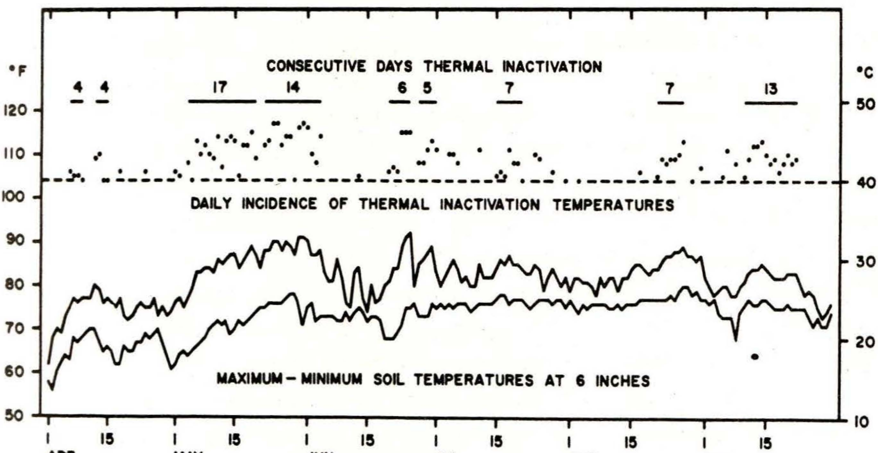

Figure 2.--April to September stump and soil temperatures in a slash pine stand at Bainbridge, Georgia. Dots represent days thermal inactivation temperatures (40° C.) were reached. Solid line above dots shows consecutive days of thermal inactivation. Lower pair of lines show maximum-minimum soil temperatures.

DISCUSSION

Since it has been shown that stump temperatures reached or exceeded the thermal inactivation level of F. annosus from April to September (Figure 1), it can be assumed that spore germination and growth of the fungus was restricted to some degree. Although stump temperatures attained thermal inactivation levels, it is evident that a low rate of infection occurred in stumps artificially inoculated (Figure 1). This would suggest that if inoculum were available some stumps might become colonized and that temperature is perhaps not the sole factor in limiting colonization of stump surfaces during the summer months. However, it is reasonable to assume that because of variation in shading by the residual stand and understory vegetation, not all stumps are exposed to the same high temperatures. Also in thinning operations, many stumps are buried under slash and are not exposed to high temperatures or natural inoculum of F. annosus. One very important factor to consider in natural infection, however, is the availability of natural inoculum. Spore trapping data taken at the time the seasonal thinning study was conducted indicated natural inoculum of F. annosus was either very low or not available during the April to September period. Even though minimal stump infection did take place during this period, it should lead to considerably less infection in the residual trees than if thinning were carried out in the winter.

Another important consideration is the effect of temperature on the colonization of stump root systems by F. annosus after the organism has colonized the stump and reached the roots. Data from this study show that soil temperatures at a 6-inch depth during the hottest part of the year never reached the thermal inactivation level for F. annosus (Figure 2). It may be assumed that root temperatures did not differ greatly from the soil. For most of the April to September period, the mean soil temperatures did not exceed the optimum growth temperature (24° C.) of F. annosus. From this information one may conclude that high temperatures did not greatly interfere with the growth of F. annosus in infected root systems in the study area. The lowest soil temperature attained during the 1-year study was 3° C., a temperature not low enough to stop completely the growth of F. annosus. Therefore, it is probable that this fungus may continue to grow in the root systems of infected trees throughout the year. This may explain why the rate of spread of this organism within a stand appears to be faster in southern regions than in northern ones where freezing temperatures may inhibit the growth of F. annosus in the roots.

In summary, stump temperatures reached or exceeded the thermal inactivation level of _F. annosus_ from April to September 1966 near Bainbridge, Georgia, and effectively limited the colonization of stump surfaces by this fungus. However, soil temperatures never reached the thermal inactivation level of this fungus and probably had little influence on its growth in infected roots.

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RESISTANCE TO INFECTION BY *FOMES ANNOSUS*

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Organisms can resist attack by pathogens. If this were not so, they would have ceased to exist. But under certain conditions barriers are breached and the pathogen advances into host tissues. It would be of considerable importance for our basic understanding of this disease relationship to identify and to determine the limitations of the resistance mechanisms available to the various host genera. Such information has wide implications in the selection and breeding of trees for resistance and it also would be extremely useful in the consideration of any control program. Unfortunately, current knowledge on this subject is very limited. I shall attempt to review briefly what is known.

There is little doubt that intact bark forms the outermost barrier to infection. The phloem of some species also contains inhibitory polyphenols (Laatsch, 1968).

Oleoresin accumulation can occur in the phloem and xylem of trees of certain genera in response to injury. The mechanism of accumulation was

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postulated (Shain, 1967). The occurrence and structure of the resin canal systems of coniferous genera were reviewed recently (Gibbs, 1968). In addition to Gibbs' (1968) extensive but circumstantial evidence that oleoresin plays an important role in resistance to F. annosus, further evidence strongly suggests that oleoresin can inhibit the fungus both chemically and mechanically. Resin-soaked pine sapwood failed to decay after a 14-week incubation period whereas similar but acetone extracted samples decayed readily. In addition, the acetone extract from the resin-soaked wood inhibited mycelial growth on malt agar (Shain, 1967). It appears that oleoresin from Norway spruce [Picea abies (L.) Karst.] also inhibits the linear growth of mycelium. I found that a 0.5% suspension in malt agar inhibited growth by about 50%. The fungus did not grow over larger droplets of oleoresin on the agar surface for the several weeks that observations were made. In light of the above it should be remembered that oleoresin in resin-soaked tissues can commonly account for 30-40% of the dry weight of these tissues.

The inhibitory substances in oleoresin are being identified. Cobb, et al. (1967) reported that a number of volatile terpenes adversely affected F. annosus moderately to severely. I tested a practical grade of abietic acid[2/], a resin acid common in spruce and pine. Resin acids are the major component of oleoresin in these genera (Mutton, 1962). The sample tested inhibited linear growth of mycelium by about 60% at a concentration of 0.25% suspended in malt agar.

Polyphenols present in the heartwood of certain species, especially the pines, convey a degree of resistance to this tissue. This aspect of resistance, particularly pertinent to the durability of forest products, was reviewed recently (Scheffer and Cowling, 1966).

In addition to the above mentioned possibilities for the host to resist infection by the presence or accumulation of preformed substances, the host may also resist by a metabolic response resulting in the production of inhibitory substances. This latter phenomenon was observed in Pinus taeda L. (Shain, 1967) and efforts are underway to determine if similar mechanisms are present in Norway spruce. Despite the extensive longitudinal invasion of heartwood in this species, penetration of living sapwood is indeed slow. This observation suggests that inhibitory substances produced in sapwood are involved in localizing radial spread of

[2/] Fluka Chemical Company

the pathogen. This suggestion has served as a working hypothesis during my work at the Norwegian Forest Research Institute[3/]. The following should only serve as a brief preliminary report.

Examination of freshly cut trees during all seasons of the year revealed that an olive to light-green zone up to 3 mm. wide surrounds the brownish core of decayed wood and separates the latter from sound sapwood. This greenish zone becomes darker in color (grey-blue to black) on the cut surface shortly after the tree is felled. Isolations and histological observations revealed that the pathogen was confined almost entirely to the brownish central core although it was present occasionally in the immediately adjacent portions of the darkened zone. Hereafter, this zone will be referred to as the reaction zone. These observations are in agreement with Björkman, *et al*. (1949) who referred to this zone as the water-stained zone in the inner part of the sapwood. Bacteria and fungi other than *F. annosus* were isolated occasionally from the reaction zone particularly in the lower part of the stem. This zone was not observed in the apical portion of the infected column where the fungus was present only in heartwood.

The color change on cut surfaces is dependent upon the presence of oxygen and it appears to be mediated by oxidative enzymes. A phenoloxidase was found localized in those ray cells of the reaction zone adjacent to sound sapwood. Evidence strongly suggests that this phenoloxidase is of host origin.

Dehydrogenase activity is lacking in the reaction zone indicating that death of host parenchyma occurs well in advance of the pathogen.

Additional features of this reaction zone include: (1) higher pH (expressed sap *ca*. pH 8.0) than other tissues (expressed sap *ca*. pH 5.5), (2) low starch content as compared to sound sapwood, (3) higher extractive content than other tissues particularly of polar extractives, *i.e.*, those soluble in 70% ethanol. [Much of this extract appears to be phenolic with ultraviolet absorption spectra characteristic of the lignans and catechins. It should be noted that this tissue is not resin soaked as is the reaction zone of pine (Shain, 1967). Resin exudation in xylem of Norway spruce and hence the possibility of resin accumulation in this species are restricted to outer sapwood. This was also observed by Gibbs (1968).], (4) higher

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[3/] Support provided by the Royal Norwegian Council for Scientific and Industrial Research.

mineral content (particularly potassium and calcium) than sound sapwood or heartwood, and (5) the moisture content of this tissue can vary in the same tree from 45-140% of dry weight. Higher moisture contents commonly occur in the lower part of the stem.

The fungus failed to grow on filter-sterilized, expressed sap from the reaction zone whereas it grew readily on similar extracts from sound sapwood, decayed wood, and sound heartwood. Since the growth of *F. annosus* apparently is limited greatly on substrates above pH 7.0 (Rennerfelt and Paris, 1952), it appears that the high pH was involved in the observed fungistasis. However, inhibition also occurred when reaction zone sap was buffered to pH 5.6, indicating an effect in addition to high pH. Attempts are being made to identify the cause of alkalinity and other constituents that may be involved in the observed inhibition.

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FOMES _ANNOSUS_: A THREAT TO FOREST PRODUCTIVITY IN THE DOUGLAS-FIR SUBREGION OF THE PACIFIC NORTHWEST?

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The Pacific Northwest region of the United States is noted for timber production. The Cascade Range divides the region into the ponderosa pine subregion to the east and the Douglas-fir subregion to the west. The threat of _Fomes_ _annosus_ (Fr.) Cooke to forest productivity in the Douglas-fir subregion of the states of Oregon and Washington is discussed in this paper.

About 11.7 million hectares (29 million acres) or 82 percent of the land in the Douglas-fir subregion is forested. Of these lands, 10.5 million hectares (26 million acres) are classified as commercial forests with a net volume of almost 3.6 billion cubic meters (130 billion $ft.^3$). Over 90 percent of the volume is softwoods; principally Douglas-fir [_Pseudotsuga menziesii_ (Mirb.) Franco], 1.7 billion cubic meters (59 billion $ft.^3$); western hemlock [_Tsuga_ _heterophylla_ (Raf.) Sarg.], 0.7 billion (26 billion $ft.^3$); and true firs (_Abies_ spp.), 0.4 billion (13 billion $ft.^3$). The annual harvest exceeds 56 million cubic meters (2 billion $ft.^3$) (U. S. Forest Service, 1965).

Past forest practices were directed primarily to harvesting the old-growth forests. Now, rapidly accelerating demands for timber products are providing strong incentives to increase forest production through intensified planting and seeding, precommercial and commercial thinnings, forest fertilization, and utilization of wood materials. Forest rotation ages have been drastically reduced and tree species previously secondary to Douglas-fir now are receiving increased attention. All factors influencing forest productivity are subject to critical examination; among these, insect and disease problems are of major importance.

Diseases cause an estimated annual impact[1/] of over 6.4 million cubic meters (230 million ft.^{3}) in the Douglas-fir subregion (Childs and Shea, 1967). Heart rots, root rots, and dwarf mistletoes are the principal causes of loss. Root rots alone result in over 0.8 million cubic meters (30 million ft.^{3}) of mortality each year and reduce potential growth by an estimated 2.0 million cubic meters (71 million ft.^{3}). Among the root rot fungi, *Poria weirii* Murr., *Armillaria mellea* (Vahl ex Fr.) Kummer, and *F. annosus* are major offenders.

Until recently, practically all root rot research in the subregion has been on *P. weirii*. Considerable concern now is developing over the potential threat of *F. annosus* and forest pathologists are faced with distributing their efforts. The impact of *F. annosus* on forest production elsewhere, especially in artificially established and intensively managed forests, indicates a potentially dangerous situation and poses serious questions as second-growth management and artificially regenerated forests in the Northwest become more common.

The fungus is present throughout the subregion, occurring primarily as a butt and trunk rot in western hemlock. It also is a common rot in dead standing or fallen trees. Englerth (1942) attributed most heart rot in western hemlock to *F. annosus*. Rhodes and Wright (1946) concluded that *F. annosus* is primarily a wound pathogen since they failed to find the fungus entering through roots except when they were injured above the ground. Wright and Isaac (1956) found *F. annosus* to be the principal fungus causing decay through logging injuries to western hemlock and the true firs; it also was associated with sunscald injuries and broken tops. Shea (1960) and Hunt and Krueger (1962) likewise noted most decay associated with logging wounds of western hemlock was due to *F. annosus*.

[1/] Annual disease impact includes disease-caused mortality, growth loss, and cull.

Susceptibility to F. annosus appears to vary greatly among the native tree species. Western hemlock and true firs are highly susceptible to invasion of logging injuries by F. annosus, whereas injuries to Sitka spruce (Picea sitchensis Bong.) seldom are invaded. Wright and Isaac (1956) found F. annosus decay in only one of 53 injured Sitka spruce and Shea (1960) in none of 79 injured trees examined. In two investigations of Douglas-fir logging injuries (Shea, 1961; Hunt and Krueger, 1962), no decay resulted from F. annosus. In California, F. annosus causes a root and butt rot of Douglas-fir but seldom kills trees directly (Wagener and Cane, 1946). Conversely, in England, Rishbeth (1951) reported young planted Douglas-fir were killed outright but older trees seemed more resistant with damage limited to root and butt rot. It appears that in present natural forests of the Douglas-fir subregion, F. annosus is likely to be a significant problem only in western hemlock and the true firs, but under certain conditions Douglas-fir is susceptible. With the establishment of plantations, strikingly different damage patterns may occur.

The general distribution of the fungus throughout many natural forest stands of the subregion provides an abundance of spore inocula. In adjacent British Columbia, Canada, Reynolds and Wallis (1966) reported spore discharge throughout the year but with notable peaks during certain seasons. A similar situation probably exists in the Douglas-fir subregion. Spore infection through injuries to western hemlock and true firs occurs readily and Driver and Wood (1968) reported a high incidence of stump infection following thinning of young western hemlock stands. Thus, increased infection associated with intensive forest management practices is likely and the potential damage as a butt and trunk rot, especially in western hemlock and true firs, is considerable.

The capacity for spread of F. annosus from infected stumps to adjacent trees in the Douglas-fir subregion is not known. Investigations now underway in British Columbia by Dr. Gordon Wallis and in Washington by Dr. Charles Driver should clarify this point. If analogies can be drawn from infected logging injuries, it seems unlikely that tree-to-tree spread of the fungus through root contact is of major significance in the present forests although it may take place. Consequently, stump infection, although occurring readily, may not be so important as elsewhere and stump treatments may be unnecessary in many instances.

If tree-to-tree spread of the fungus does occur from root contacts with roots of infected stumps or other infected trees, the timing and application of direct chemical controls to injuries and to stumps would greatly complicate the control problem. Treatment costs would be appreciably higher than elsewhere where stump treatments alone are required.

Research so far indicates chemical treatments used effectively in other areas to prevent stump infection may not be effective in the Douglas-fir subregion. Driver et al. (1968) found that dry borax, successfully used in southeastern United States to protect pine stumps, is not so effective on western hemlock stumps. Chemical analyses 12 weeks after treatment with dry borax showed practically no stump penetration or surface retention and F. annosus was isolated in culture from the stumps. Current studies by Driver suggest, however, that rainy periods at the time of application may have had an adverse effect on the treatment with dry borax; other formulations of borax or other chemicals may be needed to prevent stump infections in this subregion. Chemicals used for stump treatments elsewhere must be thoroughly tested in the Douglas-fir subregion before they can be recommended.

The widespread distribution of the fungus in forest stands of the Douglas-fir subregion raises the question of when and where effective direct control methods are needed. Moreover, a thorough study of the epidemiology of annosus root rot must be made before research results developed elsewhere can be used. It is already apparent that much of the information on F. annosus may not apply to Pacific Northwest tree species and climatic conditions. We, therefore, are faced with the complex problem of evaluating old concepts and developing new ones at a time when forest practices are rapidly changing from unmanaged to managed and from natural to artificially regenerated forests. The F. annosus problems in tomorrow's forests may differ greatly from those in today's. What we do now in forest management practices and the action taken against annosus root rot may affect greatly the future productivity of these forest lands.

Present knowledge of F. annosus in the Douglas-fir subregion of the Pacific Northwest is meager and can be summarized as follows:

1. The fungus is widely distributed throughout the natural forests where commonly it causes a butt and trunk rot of western hemlock and the true firs.

2. Spores apparently are present throughout the year but with seasonal variations in spore production.

3. Native conifers vary greatly in susceptibility to F. annosus invasion of wounds. Wounds to western hemlock and true firs, especially those in contact with the soil, are highly susceptible. Wound infection occurs less commonly in Sitka spruce and rarely, if at all, in Douglas-fir.

4. Stump infection by spores has been demonstrated in western hemlock and needs to be investigated for other important tree species such as the true firs, Sitka spruce, and Douglas-fir.

5. Tree-to-tree spread of the fungus through root contacts has not been adequately studied. It may be rare and of minor importance or it may occur more readily than heretofore believed. In either event, spread of the fungus through roots has a major bearing on the type of control program attempted.

6. If stumps and/or logging injuries need to be treated to control the fungus, materials and methods used widely elsewhere must be thoroughly tested for their effectiveness.

In conclusion, F. annosus, although not a major problem in present forests of the Douglas-fir subregion, may be a potential cause of intolerable losses in future intensively managed forests, whether naturally or artificially established. Sharp increases can be anticipated in annosus butt and trunk rot of western hemlock and true firs as injuries accompanying thinnings provide additional infection courts. Research is urgently needed to evaluate the threat of F. annosus to future forest management in the Douglas-fir subregion, to determine the significance of injuries and stumps in spread of the pathogen, and to test and develop effective control methods when, and if, needed.

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FOMES ANNOSUS IN CONIFER STANDS OF FIRST AND SECOND ROTATION

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For silvicultural as well as economical reasons Danish foresters will often prefer clear-cutting and replanting of conifer stands as a regenerative measure. This fact gives special acuteness to the problem of the *F. annosus* attacks in the second and later rotations of conifers on the same site. Since Norway spruce, *Picea abies*, is the dominating conifer in Denmark, the problem especially applies to this species, and experiments and investigations have been concentrated on the conditions appertaining to Norway spruce.

Already in the earliest Danish textbook of plant pathology (Rostrup, 1902) the following is said about *F. annosus* attacks in second-rotation conifer stands: "If a conifer stands suffers from a heavy attack, an early clear-cutting is recommendable, as the value of the stand will be diminished with each year, the increment being insufficient to make up for the destruction caused by the fungus. To regenerate such an area, where the fungus-attacked stumps are left in the soil, would only cause the new planting, especially if of conifer or beech, to be soon attacked by the disease. Where the cost permit it, it will absolutely be the proper thing to do to remove the stumps and collect the remaining roots as far as possible, and then cultivate the area for a couple of years with potatoes,

lupine, buckwheat, or what the conditions will permit, before afforestation is resumed. Where it is impossible to remove the stumps, it will be advisable to cover them with soil, by which the process of destruction is precipitated and the fructification of the fungus is prevented."

With a view to testing the efficiency of the measures suggested, an experiment with stump excavation was laid out in 1914 (Bornebusch and Holm, 1934). An approximately 40-year-old Norway spruce stand, planted on former agricultural land and heavily attacked by F. annosus, was clear-cut. The area was divided into 4 main plots, in two of which the stumps were removed by hand, whereas the stumps in the other two were left untouched. Further, the area was divided up into 79 plots and planted with various tree species, cf. Tables 1 and 2. In the period 1917-20 an annual count was taken of the number of plants killed by F. annosus and in the period 1925-33 the attacked trees in the thinnings were counted. The results are shown in Tables 1 and 2.

It appears from these tables that the removal of stumps has had no very great influence on the F. annosus attack, though it seems, for most tree species, to have had a mitigating effect. In estimating the results, one should remember that the stumps were removed by hand and that, accordingly, some bits of roots were probably left in the soil, from which the fungus may have spread.

The result did not encourage the practice in removing the stumps.

For further elucidation of the effect of stump excavation two experiments were established in the late 1950s. From these we have, so far, no definite results.

It may be mentioned, moreover, that experiments in covering stumps with soil have been made both in England (Rishbeth, 1951) and in Denmark (Paludan, 1963), but in both instances this treatment caused an aggravation of the condition.

In 1927 a questionnaire was sent to all Danish forest districts, containing a number of items concerning the prevalence of F. annosus under various circumstances, including also the incidence in Norway spruce stands of second rotation. The answers submitted may be summed up as follows (Jørgensen, Lund and Treschow, 1939): "In the great majority of cases, experiences from Norway spruce after Norway spruce are not favourable. The information is mostly to the effect that the disease occurs in a very pernicious degree in second rotation, and that continuous growing of Norway

Table 1.--The number of plants from different tree species killed by *F. annosus* in the period 1917-20 in a culture planted 1914 after heavily attacked Norway spruce and where the stumps were excavated before replanting in the half of the parcels

Tree species	Stump excavation	Number of plants: Total	Killed by *F. annosus* 1917-20	
		No.	No.	Pct.
Picea abies	-	170	7	4.1
Picea abies	+	170	0	0.0
Picea sitchensis	-	170	12	7.1
Picea sitchensis	+	170	9	5.3
Pseudotsuga menziesii	-	200	25	12.5
Pseudotsuga menziesii	+	140	13	9.3
Abies alba	-	170	0	0.0
Abies alba	+	170	0	0.0
Abies nordmanniana	-	270	3	1.1
Abies nordmanniana	+	170	3	1.8
Abies grandis	-	170	7	4.1
Abies grandis	+	170	2	1.2
Abies concolor	-	170	0	0.0
Abies concolor	+	170	0	0.0
Pinus sylvestris	-	170	6	3.5
Pinus sylvestris	+	170	6	3.5
Pinus nigra var. *austriaca*	-	100	3	3.0
Pinus nigra var. *austriaca*	+	240	3	1.3
Pinus contorta	-	170	7	4.1
Pinus contorta	+	170	7	4.1
Pinus ponderosa	-	170	1	0.6
Pinus ponderosa	+	170	0	0.0
Larix decidua	-	270	10	3.7
Larix decidua	+	70	0	0.0
Larix leptolepis	-	170	11	6.5
Larix leptolepis	+	170	9	5.3
Fagus silvatica	-	680	9	1.3
Fagus silvatica	+	680	0	0.0
Quercus robur	-	100	1	1.0
Quercus robur	+	240	1	0.4
Quercus borealis	-	170	6	3.5
Quercus borealis	+	170	5	2.9
Betula verrucosa	-	270	8	3.0
Betula verrucosa	+	170	1	0.6
Betula pubescens	-	270	14	5.2
Betula pubescens	+	70	4	5.7
Populus canescens	-	270	22	8.1
Populus canescens	+	170	11	6.5

- denotes no stump-excavation
+ denotes stump-excavation

Table 2.--Observations from the same experiment as in Table 1. The number of trees from different species attacked by *F. annosus* in thinning during the period 1925-33

Tree species	Stump excavation	Trees felled in 1925-33		
		Total	With *F. annosus*	
		No.	No.	Pct.
Picea abies	-	58	20	34.5
Picea abies	+	74	6	8.1
Picea sitchensis	-	85	61	71.8
Picea sitchensis	+	92	64	69.6
Pseudotsuga menziesii	-	124	51	41.1
Pseudotsuga menziesii	+	82	40	48.8
Abies alba	-	92	0	0.0
Abies alba	+	72	1	1.4
Abies nordmanniana	-	112	0	0.0
Abies nordmanniana	+	81	0	0.0
Abies grandis	-	114	40	35.1
Abies grandis	+	114	15	13.2
Abies concolor	-	90	12	13.3
Abies concolor	+	64	3	4.7
Pinus sylvestris	-	125	39	31.2
Pinus sylvestris	+	125	29	23.2
Pinus nigra var. *austriaca*	-	57	11	19.3
Pinus nigra var. *austriaca*	+	166	43	25.9
Pinus contorta	-	138	43	31.2
Pinus contorta	+	131	30	22.9
Pinus ponderosa	-	138	25	18.1
Pinus ponderosa	+	162	23	14.2
Larix decidua	-	227	132	58.1
Larix decidua	+	65	33	50.8
Larix leptolepis	-	128	60	46.9
Larix leptolepis	+	119	55	46.2
Fagus silvatica	-	246	16	6.5
Fagus silvatica	+	269	3	1.1
Quercus robur	-	80	0	0.0
Quercus robur	+	83	1	1.2
Quercus borealis	-	55	2	3.6
Quercus borealis	+	43	3	6.7
Betula verrucosa	-	167	21	12.6
Betula verrucosa	+	128	19	14.8
Betula pubescens	-	109	19	17.4
Betula pubescens	+	18	3	16.7
Populus canescens	-	181	81	44.8
Populus canescens	+	116	51	44.0

\- denotes no stump-excavation
\+ denotes stump-excavation

spruce through several rotations must generally be considered unwarrantable. It must be mentioned here, however, that the material obtained through the questionnaire is not very extensive, and that it suffers from certain deficiencies. Thus, there is usually no information as to the state of the area at the reafforestation and as to the health of the former stand; therefore, the estimation of the individual cases becomes rather vague, although it must be considered probable that the conditions of the cleared stands must have been tolerably good, since otherwise the owner would hardly have chosen to regenerate with Norway spruce.

Even though the experiences obtained from Norway spruce after Norway spruce are thus unfavourable, the questionnaires contained several reports of cases in which the second rotation developed nicely and reached a reasonable age before Fomes attacks of any importance occurred."

Despite the dark picture presented by this investigation, the planting of Norway spruce after Norway spruce was widely continued during the following years, because Norway spruce, even when badly attacked by F. annosus, is more profitable than other species by which it might be substituted.

In 1956 the Danish Forest Experiment Station began an investigation of soil formation, increment and state of health in Norway spruce stands of first and second rotation. The investigation was carried out in two types of soil: moraine soils in North Zealand and fat clayey soils in North and South Zealand. The results of the investigation of the moraine soils have been published (Holmsgaard, Holstener-Jørgensen and Yde-Andersen, 1961).

The investigations were made where Norway spruce stands planted after beech (first rotation) adjoined others of approximately the same age planted after Norway spruce (second rotation), and where the soil and ground conditions seemed to be the same for all stands. In all such cases a sample plot was established in each of the Norway spruce rotations, and thus the research technique is to compare, in any two sample plots, the above mentioned conditions in the two types of stand. At the same time it was checked whether Norway spruce stands containing both first and second rotation had been clear-cut at an abnormally early stage and thus withheld from the investigation; however, this proved not to be the case.

The extent of the F. annosus attack was assessed on the basis of the number of trees in which the fungus could be ascertained at stump height. From the trees in each sample plot samples were drawn aseptically by an

increment borer at stump height, corresponding to the diameter of the tree. The samples were placed in tubes with malt agar slants, and after the lapse of about a fortnight examined under a stereo microscope for F. annosus conidia. As to the reliability of this method, it should only be mentioned here that its application involves a too low assessment of the frequency of attacks in the individual sample plot, as a consequence of which also possible differences in the frequency of attacks is assessed too low. For further details, reference is made to Neger (1917) and Yde-Andersen (1964).

The incidence of F. annosus attacks on Norway spruce stands of first and second rotation respectively appears from Tables 3 and 4.

The material collected is comparatively limited and not well suited to being worked up statistically; therefore the proper thing to do would be to consider and assess it directly. It will be seen that the material falls into two groups: stands at breast-height ages of under and over 30 years, respectively.

In stands at a breast-height age of under 30 years, corresponding to an age of 38 years from seed, the majority of second-rotation stands are more heavily attacked than the corresponding first-rotation stands. On moraine gravel the frequency of attacks in the second rotation is in 7 instances higher than, in one instance equal to, and in no instances lower than the frequency of attacks in the first rotation; on the clayey soils the corresponding figures were 8, 0 and 3. The average figures for the frequency of attacks on first and second rotation, respectively, were on moraine gravel 0.3 and 9.1 and the corresponding figures for clayey soils 5.3 and 15.2. The somewhat higher frequencies found in the stands on clayey soils are partly due to the fact that the stands investigated there are, on an average, 6 years older than the stands on moraine gravel.

In stands at a breast-height age of over 30 years, the first rotation or the second rotation may show the heaviest attack. On moraine gravel the frequency of attacks in the second rotation was in 2 instances higher than, in no instance equal to, and in 4 instances lower than the frequency of attacks in the first rotation; on clayey soils the corresponding figures were 4, 1 and 3. The average figures for the frequency of attacks in first and second rotation were on moraine gravel 9.6 and 8.5 and on clayey soils 16.2 and 12.8.

There can hardly be any doubt that the recording made of the F. annosus attacks gives a pretty accurate picture of the actual state. The question

Table 3.--Parallel studies of the incidence of F. annosus attack in Norway spruce of neighbouring stands with 1. and 2. rotation. North Zealand

Sample plot	Rotation	Breast-height age	Number of trees examined		
			Total	With F. annosus	
No.			No.	No.	Pct.
Stands under 30 years					
1028	1	9	110	0	0.0
1029	2	11	103	2	1.9
1030	1	11	106	1	0.9
1031	2	18	130	51	39.2
1016	1	13	117	0	0.0
1017	2	12	110	1	0.9
1022	1	15	130	0	0.0
1023	2	15	108	3	2.8
1001	1	16	100	0	0.0
1002	2	17	96	0	0.0
1026	1	17	110	1	0.9
1027	2	15	96	2	2.1
1008	1	17	81	0	0.0
1009	2	17	79	3	3.8
1014	1	18	120	1	0.8
1015	2	18	94	12	12.8
1. Rotation		15	874	3	0.3
2. Rotation		15	816	74	9.1
Stands over 30 years					
1010	1	31	102	9	8.0
1012	2	31	98	11	11.2
1011	1	31	110	8	7.3
1013	2	36	61	3	4.9
1018	1	32	93	8	8.6
1019	2	32	103	4	3.9
1006	1	33	109	14	12.8
1007	2	31	97	7	7.2
1004	1	53	94	1	1.1
1005	2	42	51	5	9.8
1024	1	59	97	18	18.6
1025	2	53	142	17	12.0
1. Rotation		40	605	58	9.6
2. Rotation		38	552	47	8.5

Table 4.--Parallel studies of the incidence of F. annosus attack in Norway spruce of neighbouring stands with 1. and 2. rotation. South Zealand

Sample plot	Rotation	Breast-height age	Number of trees examined: Total	With F. annosus	
No.			No.	No.	Pct.
Stands under 30 years					
1057	1	15	120	0	0.0
1058	2	23	106	3	2.8
1039	1	19	159	1	0.6
1040	2	19	117	9	7.7
1055	1	19	137	2	1.5
1056	2	19	113	0	0.0
1041	1	20	117	0	0.0
1042	2	21	191	85	44.5
1037	1	20	129	2	1.6
1038	2	22	120	22	18.3
1045	1	20	86	0	0.0
1046	2	22	97	14	14.4
1065	1	21	96	0	0.0
1066	2	23	99	13	13.1
1033	1	23	86	3	3.5
1034	2	23	142	25	17.6
1069	1	23	96	18	18.7
1070	2	24	101	31	30.7
1043	1	24	146	26	17.8
1044	2	22	119	18	15.1
1067	1	25	91	13	14.3
1068	2	25	85	3	3.5
1. Rotation		21	1263	65	5.3
2. Rotation		22	1290	223	15.2
Stands over 30 years					
1063	1	27	96	5	5.2
1064	2	33	90	4	4.4
1035	1	31	110	65	59.0
1036	2	26	203	98	48.2
1047	1	35	101	39	38.6
1048	2	35	88	15	17.0
1049	1	37	119	4	3.4
1050	2	31	89	3	3.4
1059	1	41	93	1	1.1
1060	2	38	121	5	4.1
1053	1	42	116	5	4.3
1054	2	39	85	6	7.1
1051	1	56	87	7	8.0
1052	2	46	99	8	8.1
1061	1	57	89	9	10.1
1062	2	49	96	10	10.4
1. Rotation		41	811	135	16.2
2. Rotation		37	871	149	12.8

is, however, whether the picture obtained may be used straight away as a basis for a prognosis, or it may only be looked upon as historical experiences of doubtful relevance to the future.

Before answering this question, we must consider a number of circumstances. About the soil condition in the stands investigated it may be said that between the first- and second-rotation sample plots, either before or after their being planted with Norway spruce, no differences with a clear tendency towards one side was provable, marked enough to be expected, according to our knowledge, to have involved changed conditions for the occurrence of F. annosus. The soil investigations furthermore showed that the pH measured in KCl varied around 3 at the depth of 10 cm. and, with a few expections, around 4 at the depth of 110 cm. This fact may to some degree explain why the frequencies of attacks found are generally so moderate, because the spreading of the fungus from tree to tree and from stump to stump is restrained by low pH values (Rishbeth, 1951; Wallis, 1960).

The first-rotation stands investigated constitute a fairly homogeneous body; they have all been planted on areas that were covered with beech immediately before being planted with Norway spruce. As to thinning intensity and exposure to infections in other respects, conditions were, however, not even for the investigated stands, cp. what is said under second-rotation stands.

The investigated second-rotation stands constitute in respect of health a heterogeneous body. No information is available as to the distribution of the F. annosus attacks in each single stand of the first-rotation stands that were succeeded by the second-rotation stands, or even as to whether the state of health in the first rotation varied quite accidentally from stand to stand. On the other hand, it is suspected that the intensity of the F. annosus at the clear-cutting of the old first-rotation stands was to some degree dependent on the period in which they were felled. It is probable that the first-rotation stands regenerated fifty years ago were not so heavily attacked by F. annosus. This assumption is based, partly, on the fact that the thinning practice was quite different from those practiced now. The first thinning was made at a much later stage in the lives of the stands, the intervals between thinnings were longer, and the grade of thinnings were longer, and the grade of thinning lighter, cp. Dalgas (1920); finally, the thinnings were usually carried out in the winter. The mentioned circumstances will all have a limiting effect on the number of

stump-surface infections by airborne F. annosus spores (Henriksen and Jørgensen, 1952; Yde-Andersen, 1961). To this comes further that the F. annosus spore-density in the air was probably far lighter then than it is now, i.a. because the host-plants of the fungus were fewer, since the total conifer area was small. Different conditions apply to the first-rotation stands regenerated within the last 30 years. These stands were thinned earlier, heavier and more frequently, cp. Møller (1933), and the thinnings were often carried out during the summer, and thus it must be presumed that they have been more heavily attacked by F. annosus.

For the reasons stated it must be assumed that of the second-rotation Norway spruce stands comprised by the investigation, the younger were planted after Norway spruce stands which had to limited extent been attacked by F. annosus, whereas the older were planted after stands which were almost free from attacks. As the latter situation is not likely to occur in the future, cp. the observed frequencies of attacks on the first-rotation stands, the frequencies of attacks observed cannot without corrections be expected to forecast a future typical course of the disease in second-rotation Norway spruce stands on the localities concerned. An estimation of the probable development of the disease in the second-rotation stands should much rather be based on the figures from the younger stands.

On the assumption that the present felling method will be used unchanged in the future, and that no stump-surface treatment will be employed, the results obtained from the younger stands may form the basis of the following (uncertain) prognosis of the course of the F. annosus attack on Norway spruce stands in the areas investigated.

In first-rotation Norway spruce stands, a F. annosus attack will not occur until approximately 5 years after the first thinning, subsequently the attack will increase in extent to comprise at rotation age between 10 and 20 percent of the trees.

In the second rotation there will be a risk of a F. annosus attack before the first felling, and already at the first thinning it may be expected that between 5 and 10 percent of the trees have been attacked. In that case the attack will increase in intensity to comprise at rotation age between 30 and 50 percent of the trees.

As, thus, there is a risk of early and comprehensive F. annosus attacks on second-rotation Norway spruce stands, an experimental treatment of the stumps from the clear-cutting with sodium nitrite before replanting with

Norway spruce was carried out simultaneously with the stump-clearing experiments mentioned. The background of these experiments is the results obtained from stump-clearing experiments in England (Forestry Commission, unpublished), from which it seems to appear that it is especially from the stumps after clear-cutting that infections come to the new stand.

Finally it should be mentioned that investigations are now being made to elucidate the spreading of the fungus from tree to tree by the growth of mycelium on and in the roots. These investigations include, i.a., a study of the root-surface flora of Norway spruce. The investigations are carried out in two different types of stand. Stands in which we know from experience that *F. annosus* is spreading quickly from tree to tree, *e.g.*, stands on former agricultural land, and stands in which the disease is spreading only slowly, *e.g.*, stands on moraine gravel with a low pH. The composition of the root-surface flora in respect of species and its bearing on *F. annosus* is investigated, and an attempt is made at elucidating a possible connection between these factors and the spreading of the disease. In the investigations a technique is employed which is a further development of the one suggested by Harley and Waid (1955). These investigations are still at a too early stage for results to be published.

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EXTENSION AND CAUSE OF BUTT ROT IN A SPRUCE PLOT

IN REINHAUSEN (LOWER SAXONY, GERMANY)[1/]

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To find the cause of butt rot in living spruce (Picea abies), several test areas were chosen. The individual trees were analyzed on properties and butt rot. The results of one area with 177 trees are reported. The results do not indicate that dominant or suppressed trees are particularly attacked by butt rot fungi.

Fungi were isolated from different spots of the decayed wood and identified by culture characters. Seventy-two percent of the living trees showed a butt rot. Fomes annosus could be detected in 76 of the 106 stems. In 42 trees the rot, which came through the root, was caused by other fungi. In 12 trees it could be shown that in addition to Fomes annosus, a second rot-causing fungus had independently invaded the tree from the roots.

Of the Basidiomycetes only Stereum sanguinolentum and Trametes odorata were detected. The occurrence of the Ascomycete Coryne sarcoides is interesting. This fungus was demonstrated in 8 cases of butt rot.

[1/] The observations will be published in detail and distributed shortly.

Some observations on the relation of the diameter of the trunk to the diameter of the butt rot and to the vertical extension of the rot are reported.

PANEL SESSION 1--INFECTION PHENOMENA

J. Rishbeth (UK), Chairman, C. S. Hodges (USA),
and L. Shain (USA)

INTRODUCTION

For stump infection via the cut surface the type of tree is important, stumps of pines and Douglas-fir, for example, being readily colonized; stumps of hardwoods such as birch are also sometimes colonized. Recent work by Ross (southern USA), Punter (Canada) and Kallio (Finland) for instance, elucidates further the conditions affecting spore production by _F. annosus_ and its infection of stumps. Other modes of stump infection include invasion from tissues already occupied at the time of felling, as with butt- and root-rots, and entry from newly infected stump roots. Entry through wounds on stems and roots are important in some areas such as the northwestern USA, where western hemlock and the true firs are often decayed as a result. In Britain, infection of brashing wounds on suppressed pines may lead to the development of root-infection foci. Establishment of _F. annosus_ on roots through root contacts or grafts is generally the most important means of infection, however. Modes of resistance to infection are believed to include meristematic activity, mobilization of oleoresins (especially in pines) and production of antifungal substances such as polyhydroxyphenols.

DISCUSSION

Rishbeth: What are the effects of very cold continental winters?

Punter: Colonization of stumps is not influenced by their exposure to temperatures of -20° C. Wounds seem just as receptive during cold winters as in early spring.

Laine: In connection with winter infections it is relevant that F. annosus spores will travel over greater distances than can be covered in one day.

Rishbeth: Spores seem to travel over longer distances in moist air.

Ricard: Have minimum temperatures been established as regards germination?

Cowling: Slightly below zero.

Burdekin: On malt agar?

Cowling: Yes.

Krstic: Is there any difference between races of F. annosus in relation to temperature?

Cowling: Tests show no differences.

Marx: Has any relation been detected between air and stump temperatures?

Hodges: This is better known for warmer conditions; in the southern USA the temperature at the stump surface may be considerably higher than the surrounding air.

Rishbeth: Are trees frozen right through?

Björkman: Yes. In northern Sweden trees are normally frozen right through during the winter, and sometimes also the soil is frozen to a considerable depth. In the southernmost parts of Sweden where sporophores of Fomes annosus occur above ground trees or soil are seldom deeply frozen. The fungus can certainly survive in trees at very low temperatures.

Houston: Has anybody frozen spores?

Shea: I know of no observations, but infections may occur even under 6 inches of snow.

Rishbeth: What happens during the winters in Denmark?

Yde-Andersen: In periods of frost (mean temperature of +1° C.) no spores are released and no infections occur. Production starts again in spells of mild weather.

Kallio: In Finland F. annosus spores were detected by exposing cross-sections of spruce when the temperature was as low as -20 to -30° C. and snow had been lying on the ground for a month.

Hodges: Evidently freezing has no influence.

Punter: Perhaps thawing destroys the spores.

Laatsch: Temperature not only influences spores but also the resistance of trees to infection.

Rishbeth: Is there a risk that healthy trees may become infected through roots as a result of being blown over?

Marx: Certainly healthy trees with shallow roots, as over a hard pan, can easily be blown over. What are the smallest feeder roots to become infected: 3-4 mm.?

Roll-Hansen: Fungi can penetrate through roots smaller than 1 cm. Fomes annosus has never been found entering through small wounds in small trees.

Björkman: Wounds (2-3 cm.) caused, e.g. by tractors, will always be dangerous. Smaller wounds on smaller roots are unimportant.

Hodges: Infections on stump roots are possible even where the stump surface is well protected.

Yde-Andersen: Wounds on the stem just above ground level have not been found infected with F. annosus. The fungus was never found in roots of suppressed trees.

Shea: With Abies, infections occur through wounds: investigations are in progress.

Yde-Andersen: In Europe Abies seem the only conifers which are really resistant to butt rot. For any given species environment may affect resistance.

Björkman: Abies alba is very resistant even when other species in the same environment are infected.

Moriando: By contrast A. alba in Italy is heavily attacked, leading to killing, especially in the Apennines.

Krstic: Spruce is heavily attacked in Yugoslavia.

PANEL SESSION 2--CONTROL

C. S. Hodges (USA), Chairman; J. Rishbeth (UK),
and B. J. W. Greig (UK)

INTRODUCTION

The methods of control for *Fomes annosus* can be divided into several categories: (1) chemical, (2) biological, (3) environmental, and (4) manipulation of forest management practices. Research on chemical control began with Rishbeth's work which showed freshly cut stump surfaces to be the primary means of entrance of *F. annosus* in a previously non-infested stand. Numerous chemicals have since been tested and recommended for use, including creosote, ammonium fluoride, sodium nitrite, borax, urea, and ammonium sulfamate.

Some materials, like creosote, have proven ineffective in certain instances; others, like borax and sodium nitrite, continue to give effective control. Even these chemicals have some disadvantages. Borax tends to preserve the stump for several months and direct root infection by *F. annosus* may occur because the roots are not invaded by antagonistic microorganisms. Sodium nitrite may be somewhat toxic to animals if proper precautions are not used.

An ideal stump treatment should be easily available, cheap, easy to apply, safe, and above all effective, not only in preventing infection at the stump surface, but in killing the stump rapidly allowing its colonization by organisms antagonistic to F. annosus.

Most of the work in the past on biological control has been done with Peniophora gigantea and Trichoderma. P. gigantea gives very effective control of F. annosus when inoculated onto pine stumps but it does not colonize spruce stumps very well. In the southern United States it appears to be effective in limiting stump infection under natural conditions and means should be devised to encourage its sporulation in the forest. Trichoderma spp. do not appear to be as effective in competing with F. annosus.

In the southern United States, temperatures at the stump surface often reach levels lethal to F. annosus (40° C. for 2 hours in wood) for several months during the year. By thinning during this period, the amount of stump infection is greatly reduced.

Also in the southern United States, prescribed burning before thinning, which is a common management practice, has been shown to reduce the amount of F. annosus in the residual stand. Burning does not have any measurable effect on chemical and physical properties of the soil but does result in the increase in the number of Trichoderma propagules isolated from the soil. Other organisms do not appear to be significantly influenced.

Other management practices which have been proposed for reducing losses from F. annosus include use of mixed stands, wider spacing, delayed thinning or not thinning at all, and use of resistant species such as hardwoods.

In addition to preventing entry of F. annosus into a previously non-infested stand, some attention should be given to preventing spread of F. annosus in established centers. This is especially important on high-value areas such as watersheds, recreational sites, and arboretums.

DISCUSSION

Houston: How toxic is sodium nitrite?

Burdekin: Toxic levels for cows, sheep, and other domestic animals are in the range of 90-100 mg./kg. fresh weight. Animals would probably not get this much by licking the stumps. They may get enough from containers of the material left in the woods. Toxicity is not a problem of sufficient importance to limit the use of sodium nitrite as a stump treatment chemical.

Houston: What happens to sodium nitrite when it is applied to the stump?

Punter: It is used as a nitrogen source by many microorganisms and is rapidly utilized. It has no residual danger.

Laatsch: How many kilograms of sodium nitrite are used per hectare?

Punter: The material is applied as a 10% solution, averaging about 50 ml. per stump. The amount per hectare would depend on the number of stumps treated.

Laatsch: In the soil the nitrite would be converted to nitrate. In Germany there is a maximum allowable amount of 50 mg. nitrate per liter in the ground water. In some agricultural areas the ground water contained nitrates in excess of this amount.

Rishbeth: Care must be taken in water catchment areas to use alternative materials such as urea.

Burdekin: Nitrate or nitrite also interferes with the chlorination process in water.

Hodges: The eventual breakdown of urea is to nitrite and nitrate. Why can it be used in catchment basins?

Rishbeth: Stumps contain large amounts of urease which converts urea into ammonia and carbon dioxide. There is a strong smell of ammonia on stumps treated with urea.

Punter: Urea is broken down to nitrite and nitrate in soil under certain conditions. I do not know what happens on the stump.

Hodges: What are the techniques for evaluation of chemicals such as urea and sodium nitrite which encourage the growth of molds which might mask the presence of *F. annosus*?

Greig: Most of our trials are short-term. We apply a number of various chemicals to pine or Sitka spruce stumps. The stumps are then inoculated with spores of *F. annosus*. After various periods of time the stumps are assayed for the amount of *F. annosus* and other microorganisms.

Rishbeth: Stump surface evaluation is followed up by root sampling. This is an important point because the amount of *F. annosus* penetrating to the roots is crucial to infection.

Punter: For complete evaluation of stump treatment chemicals it is necessary to inoculate roots as well as the stump surface. This is

especially important for a stump treatment to be used in an area where F. annosus is already present. Chemicals which have a preservative effect may actually enhance the capability of F. annosus to invade from below because of exclusion of all organisms from the root surface.

Hodges: What should the recommendation be for stump treatment in a second thinning when F. annosus is already present?

Houston: In the northeastern United States the recommendation is to treat with urea or borax.

Rishbeth: Treatment of the stumps with certain chemicals may make the situation worse. It may be best to leave the stumps alone. The best chemicals would be those which lead to the colonization of effective competitive fungi (e.g., ammonium sulfamate which encourages P. gigantea). Direct inoculation with such fungi would also be effective.

Hodges: The recommendation in the southern United States is to definitely not use borax in such stands. Our recommendation is for summer thinning. The stump cambium dries quicker when trees are cut in the summer, thus allowing the stump and roots to be colonized quickly by other microorganisms. Further stump surface infection is also prevented by the high temperatures during the summer.

Marx: What is the lethal temperature for P. gigantea?

Hodges: It is not known. It does have a slightly higher optimum than F. annosus.

Marx: Is the absence of stump infection during the summer in the southern United States due entirely to temperature per se?

Hodges: 1. It is related to high temperature per se. 2. It is related to the growth of other organisms at temperatures which retard F. annosus. 3. It is related to the rapidity of death of stumps and subsequent colonization by competitive organisms. 4. It is related to the production of low numbers of spores during this period.

Cowling: Has a better commercial source of P. gigantea inoculum been devised?

Rishbeth: Work is proceeding on a liquid formulation but no steps have been taken to produce it in bulk.

Marx: How does prescribed burning influence the natural production of P. gigantea inoculum?

Hodges: Burning would reduce the inoculum level of P. gigantea. However, if burning is successful in decreasing the amount of F. annosus, P. gigantea would not be needed.

Hodges: How expensive is stump treatment?

Greig: In England labor costs vary between 0.5 and 1.0 penny per stump.

Hodges: Who does the treatment and how is it done?

Greig: Treating is done by the man who fells the tree. The chemical is applied immediately after felling with a brush or a plastic squeeze bottle.

Punter: In Ontario the cost of treatment in the first thinning is 55 cents per cord.

Hodges: Has anyone used sodium nitrite in the powdered form as borax has been used?

Punter: No. It has the disadvantage of being a fire promoting agent in the solid form. It is also a strong oxidizing agent.

Rishbeth: Polybor chlorate was used effectively as a powder in England but the Forestry Commission preferred a solution.

Marx: What was the basis for choosing chemicals for testing as stump protectants?

Rishbeth: The choice was rather arbitrary in the beginning. The failure of creosote prompted studies on the mechanics of stump infection and the effect of chemicals on toxicity to F. annosus and selectivity for competitive microorganisms. Punter then systematically studied various compounds.

Punter: I examined a wide range of common inorganic ions. Heavy metals were in general broadly fungitoxic. Nickel was interesting in that it selected for Trichoderma, Penicillium, and Calcarisporium. Sodium nitrite was chosen because it was toxic to F. annosus, rapidly killed stump tissues and encouraged other microorganisms.

Marx: Has sugar been used as a stump treatment?

Rishbeth: Twenty percent solutions of sucrose and dextrose were ineffective.

Houston: In the northeastern United States, borax applied at a heavy rate (1/4-inch thick) on stumps resulted in some phytotoxicity in residual trees.

Shea: Commented on the importance of considering "flashback" toxicity to residual trees in testing stump treatment chemicals. This may be especially important in species where root grafting is common.

Rishbeth: In early work with ammonium sulfamate some phytotoxicity to residual trees was noted. Agreed with Shea that phytotoxicity be considered in evaluating stump treatment chemicals.

Hodges: What other chemicals are commonly used for stump treatments?

Houston: Urea is recommended in the northeastern United States.

Yde-Andersen: Creosote gave excellent results when used in a spruce plantation several years ago.

Yde-Andersen: Is there contradiction between statements by Rishbeth who indicates that boron kills stump tissues and by Hodges who indicates that borax keeps stumps alive?

Rishbeth: The answer is in the solubility of the boron compounds used. Disodium octaborate (polybor) is soluble in water to the extent of about 50%, whereas borax is soluble to about 9.6%. High concentrations of polybor gave rapid killing of stump tissues, especially in spring treatments. It also promoted stump colonization by *Botrytis cinerea*. This fungus also grew at rather high concentrations in Petri-dish culture. The difference in activity between polybor and borax is probably one of concentration. By using a color test developed by CSIRO in New Zealand, which is sensitive to about 0.01%, boron was found to move in 5 weeks to the origin of the lateral roots, at least 5 inches below the stump surface.

Björkman: Why do pine and spruce stumps differ in their colonization by *P. gigantea*?

Rishbeth: The underlying reasons are unknown but they are of fundamental importance. Many fungi show a definite affinity for certain substrates, probably due to the presence or absence of some nutritional or other factor.

Shain: Suggests that some isolate of *P. gigantea* be found that will readily colonize spruce stumps.

Rishbeth: In the laboratory, isolates from spruce stumps showed no greater facility to colonize spruce than pine isolates. Only British isolates were used. It may be possible to find an isolate of *P. gigantea* that would effectively colonize spruce stumps.

Hodges: Discussed the use of Trichoderma as a biological control agent for *Fomes annosus*. Various species of Trichoderma react differently with

F. annosus when paired on agar plates. Some overgrow F. annosus rapidly; some only slowly overgrow F. annosus. Others make contact with the advancing margin of F. annosus and further growth of both organisms ceases and finally the mycelium around the periphery of the F. annosus colony lyses. Two isolates of Trichoderma were overgrown by F. annosus. If freshly dug root sections are inoculated on one end with Trichoderma and on the other end with F. annosus and the fungi allowed to grow toward one another, both will cease growth upon meeting. This is true regardless of the species of Trichoderma involved.

Malla: In antagonism experiments using root pieces, how long are the experiments run?

Hodges: In most cases 2 or 3 months after the test fungi, which were inoculated on either end of the root piece, had grown together in the root. Commented that blue stain fungi make a good model for this kind of study because you can visually see how far this fungus has grown and where the two organisms have met in the root piece.

Shain: Are the F. annosus colonies that are overgrown by Trichoderma dead?

Hodges: No. At least not for several weeks.

Malla: I have also noted that different isolates of Trichoderma have different rates of growth and effects on F. annosus.

Hodges: Do you feel that Trichoderma is effective in preventing growth of F. annosus in wood?

Malla: It does not prevent growth but slows it down.

Krstic: There are many other organisms besides Trichoderma and Peniophora which might be effective antagonists of F. annosus.

Hodges: Tests with more than 100 isolates of fungi, bacteria, and Actinomycetes in plate cultures against F. annosus gave the same range of reactions mentioned earlier for Trichoderma. Only some bacteria and Actinomycetes and a very few fungi, notably a Penicillium sp. and a Fusarium sp. produced an inhibition zone. With the exception of Trichoderma, all fungi which overgrew F. annosus were other Basidiomycetes.

Krstic: Do you think Actinomycetes could be used to protect roots?

Hodges: Roots, yes. Actinomycetes do not appear to be common colonizers of the stump surface.

Malla: Do you find bacteria and fungi which kill spores or suppress spore germination by F. annosus in vitro?

Hodges: Yes, a number do.

Rehfuess: Are the interactions between F. annosus and other microorganisms dependent on environmental factors?

Hodges: We have no definite data on pH but temperature has a definite effect. The optimum temperature for F. annosus is about the same as many associated microorganisms but it will usually grow at lower temperatures. Low temperatures would thus enhance colonization by F. annosus. High temperatures would be detrimental.

Rishbeth: Agrees, especially in relation to G. virens which is greatly inhibited at low temperatures.

Hüppel: We have had the same experience in Sweden. When Trichoderma and F. annosus are grown together at low temperatures, F. annosus is unaffected. At the optimum temperature for Trichoderma, F. annosus is overgrown.

Houston: Can roots already colonized by Trichoderma be inoculated with F. annosus?

Hodges: No. Fomes annosus will not infect such roots. On the other hand, if you inoculate roots infected with F. annosus with Trichoderma you get some replacement of F. annosus. If stumps colonized by F. annosus are invaded by Trichoderma, the Trichoderma will follow in the area of the stump already colonized by F. annosus but will not grow through the advancing zone of F. annosus. P. gigantea will do this, however, and completely replace F. annosus in the stump.

Houston: What is the reason for this?

Hodges: I have no idea.

Marx: The advancing front of F. annosus could be physiologically different from the area already colonized.

Hodges: When the area behind the advancing front of F. annosus is colonized by Trichoderma, this effectively blocks subsequent colonization by P. gigantea. This is the reason that a greater number of stumps treated with ammonium fluoride had F. annosus (see paper by Hodges).

Rishbeth: If you inoculate non-treated stumps with Trichoderma, quite large dosages do not prevent later infection by F. annosus. Practical use of Trichoderma without stump treatment is almost nil.

Marx: Has Gliocladium virens been tested as a stump treatment?

Rishbeth: No, but I suspect it would be similar to Trichoderma in its effectiveness. It may not be able to colonize freshly cut stumps.

Hodges: The most common species of Gliocladium found in wood is G. deliquescens. In root pieces and on agar plates it reacts with F. annosus the same way as Trichoderma spp., which rapidly overgrow F. annosus.

Yde-Andersen: Is a fungus known which might be used to inoculate spruce stumps that would be as effective as P. gigantea on pine stumps?

Rishbeth: No.

Yde-Andersen: There was some work in Poland which showed that certain lichens on spruce stumps may prevent infection by F. annosus. Has anyone here done any work with lichens?

Rishbeth: No, but it is an extraordinarily interesting field.

Rishbeth: With Norway spruce, reasonable success has been obtained with P. gigantea, Polyporus adustus, and Stereum sanguinoletum. The last, of course, should be dismissed from further consideration. These fungi grow much slower in Norway spruce than in pine. They are not effective in Sitka spruce. We may have to consider inoculation combined with stump treatment to facilitate colonization.

Shain: Would stump inoculation with Scytalidium be effective on spruce?

Ricard: I am reluctant to comment at this time because this fungus has been tested in the field only against P. carbonica in poles.

Hodges: Is it correct that all isolates of Scytalidium do not produce the antibiotic?

Ricard: The anitbiotic is inducible and is produced only when it is grown in a competitive environment. All Basidiomycetes will not induce production of the antibiotic. F. annosus, F. subroseus, Poria carbonica, and P. weirii will induce the antibiotic in vitro. Poria vaporaria is not as effective in triggering release of the antibiotic.

Marx: How do you determine that the antibiotic is induced?

Ricard: Scytalidium has the advantage of releasing a yellow pigment (which is not the antibiotic) when it is producing the antibiotic. This is used to determine if an isolate is producing the antibiotic. Most isolates from

nature lose their ability to produce the antibiotic after several transfers. When placed in a competitive environment again, the antibiotic, and the pigment, is produced. This phenomenon has also been reported for Aspergillus versicolor.

Hodges: Asked Houston to discuss work he has done on preventing further spread of F. annosus in established centers.

Houston: In areas such as arboretums, seed orchards, watersheds, etc., where the value of trees far exceeds their worth for wood, considerable expenditures can be justified for stopping the spread of F. annosus in established centers. Because of this a study was initiated to test one means of preventing such spread. The premise under which the study was conducted was that if the center could be successfully delineated, and a band of roots killed around the center by soil fumigation, these roots would be rapidly colonized by soil fungi and the spread of F. annosus through the roots would be stopped. Studies were conducted in red pine stands on various soil types using methyl bromide and Vapam. Methyl bromide proved to be the most effective fumigant. Approximately 1 pound of the fumigant was used for injection at a depth of 20 inches into 8-9 holes spaced at 1-foot intervals. Roots were killed for approximately 2-1/2 feet on each side of the fumigation line. The dead root areas were colonized by microorganisms. Some cambial damage was noted on the stems of the trees when fumigation was done in the spring. No such damage was noted following fall fumigation. Successful fumigation could be done at very low temperatures. It is too early to determine if tree vitality will be affected by the partial root kill. To determine the effect of fumigation on roots infected by F. annosus, stumps in various stages of decay were subjected to fumigation under polyethylene covers. In well rotted root systems, F. annosus was killed. In roots on stumps of trees that had just died or had been living but bore conks, F. annosus was not killed. This was primarily because of resin impregnation in the latter two cases. The fungus could be recovered only where the roots were impregnated with resin. At present it is not known how fast new roots will grow across the barrier. It is also not known if further treatment will be necessary.

Shain: How long does it take the roots to die?

Houston: It is not known. Observations are made after 1 year because of the difficulty in determining exact time of death.

Shea: How long will F. annosus remain viable in resin-impregnated roots? Poria weirii may remain alive for more than 50 years in Douglas-fir roots.

Houston: I have found it in roots for up to 10 years.

Hodges: In the southern United States, F. annosus will remain alive in resin-impregnated wood for at least 5 years.

Shain: Fomes annosus can utilize resin in wood at a very slow rate and would thus be able to stay alive in such roots for long periods.

Hodges: Is trenching an effective method of restricting spread of F. annosus?

Rishbeth and Yde-Andersen: It is not effective.

Hodges: How does one delineate an infection center?

Houston: This is a big problem. The factors I consider are when the stand was thinned and observed rates of spread in a given area. A safety factor of an additional row of trees is then added. Another consideration in the northeastern United States is that most infected living trees bear conks.

Hodges: What silvicultural means of control for F. annosus have been tested?

Greig: In England some experimental plots were established in pine 8-10 years ago to study effect of delayed thinning and lack of thinning on losses to F. annosus. Evaluation will be made of both losses from F. annosus and of economic returns.

Hodges: How about the use of mixed stands?

Yde-Andersen: Where Japanese larch and moutain pine were used as nurse trees with Norway spruce, very heavy attacks of F. annosus were experienced when the nurse trees were removed. When they were not removed no attacks occurred. Mixed stands of silver fir, which is resistant to F. annosus, and Norway spruce have not been successful on heathlands because the fir does not grow well and is heavily damaged by roe deer.

Björkman: In Sweden, spruce has been replaced with other species; wherever possible with resistant species such as white oak. In southern Sweden, a rotation of birch or beech between two rotations of spruce, reduced F. annosus attack in the second spruce rotation.

Yde-Andersen: Concerning the use of thinning practices, in Norway spruce stands given light to heavy thinning, attack by F. annosus is very heavy. There is much less damage in stands receiving very heavy thinning. The reason for this is not known.

Houston: The same is true for stands in the northeastern United States. The reason may be some changes in soil and atmospheric conditions following removal of large numbers of trees.

Rishbeth: Very heavy thinning may not always result in decreased incidence of F. annosus. With pine the residual trees could theoretically benefit from a number of factors including better soil moisture which would promote production of resin. On the other hand, heavy thinning may result in such a large inoculum level in the soil that the stand will disintegrate.

Marx: Do the stumps decompose more rapidly after heavy thinnings?

Houston: I don't know. The more rapid drying of the upper portion of the stump may result in slower decomposition.

Roll-Hansen: In cool, wet periods the top of the stump decays first; whereas the roots decay first during warm, dry periods.

Hodges: More critical evaluation of the intensity of thinning on attack by F. annosus may be a fruitful area for additional research.

Burdekin: Has anyone considered methods of preventing infection by F. annosus through wounds?

Shea: Nothing has been done. This is an important factor in western hemlock where logging wounds are common. Some important considerations for treating wounds would be type of chemical, phytotoxicity, timing of treatments, and penetration.

PANEL SESSION 3--THE SPREAD OF THE FUNGUS

D. H. Marx (USA), Chairman, A. Yde-Andersen (Denmark),
J. Rishbeth (UK), and A. Hüppel (Sweden)

INTRODUCTION

Marx: The topic of this mornings discussion is the spread of *Fomes annosus* in and on roots with particular reference to the interaction with other microorganisms both in the soil and root surfaces.

Yde-Andersen: Mycelium of *F. annosus* has been found in forest soil but only under sporophores, on infected stumps, or in direct connection with infected roots. Attempts to reisolate the pathogen from soil-borne mycelium have been unsuccessful. Several workers have attempted to grow *F. annosus* in sterile and non-sterile soils. Hiley successfully grew the fungus on heat sterilized and autoclaved soil but not on non-treated soil. Fomes was not able to grow on these soils following inoculation of the soil with a bacterium. He concluded that the pathogen could grow in non-sterile soil but only in the absence of bacteria. Hopffgarten attempted to grow the fungus from wood pieces into non-sterile soil but without success. In most cases he could not reisolate the fungus from inoculum incubated in non-sterile soil due to the presence of other fungi colonizing the inoculum. He concluded that *F. annosus* was not able to live in non-sterilized soil due to the presence of antagonistic soil fungi.

Saschue, Rennerfelt, Braun and Roll-Hansen independently attempted similar experiments with F. annosus and came to the same conclusion. Saschue reported Trichoderma viride to be highly antagonistic to F. annosus. Hoffman was able to grow F. annosus in non-sterilized soil but only in test systems very unlikely to occur in nature. We must conclude from these results that F. annosus is not able to grow vegetatively in natural forest soil due to the presence of antagonistic microorganisms. Accordingly, it is unlikely that the pathogen can spread from tree to tree by means of mycelial growth in soil. Workers in Sweden could not find differences in the soil microflora of stands in which the pathogen spread rapidly or in stands where the pathogen did not spread. Currently, research in Denmark is aimed at determining the difference in the root surface microflora of Norway spruce stands on agricultural lands in which F. annosus spreads rapidly and in stands of low pH in which the pathogen spreads slowly. Results are not available at this stage of the investigation.

Rishbeth: Manka has analyzed the microflora of roots and adjacent soil and their relationship to F. annosus attack. He used roots not more than 1 mm. in diameter and a modification of Harley and Waid's direct plating-technique. Fungal isolates were examined in pure culture for antagonism against F. annosus and scored according to their influence on the pathogen and their frequency of isolation from the soil or root surface. In stands heavily infected by F. annosus he found only a few scores indicating high antagonistic influences against the pathogen, whereas the opposite was found where Fomes attack was less. Differences were found when samples were assayed in spring and in autumn. He concluded that the functional structure of the fungal community influenced F. annosus in soil and roots.

Hüppel: We have recently found in Sweden, as Marx has in the United States, that in antagonism plate culture many ectotrophic mycorrhizal fungi can inhibit growth of F. annosus. The degree of inhibition varies between species of symbionts and also between strains of the same species. As example, all five strains of Boletus variegatus tested inhibited the pathogen with inhibition zones varying from 3 to 24 mm., whereas strains of B. luteus did not cause inhibition. Other symbiont species which strongly inhibited F. annosus were Clitocybe nebularis and Tricholoma nudum. Amanita rubescens and Cenococcum graniforme were ineffective against the pathogen. Three isolates of B. edulis caused inhibition zones of 2 to 12 mm. while two were ineffective. Similar results were found with isolates of Monotropa. Tests were made to determine the effect of the antagonistic symbionts on

F. annosus infection of Norway spruce and Scots pine seedlings. Seeds were planted on the surface of soil previously prepared by layering either 2 1/2 inches of barley permeated with B. bovinus, F. annosus, or fungus-free barley grains (control layer). The order of layered inoculum which were separated by perlite was used as treatments and all 16 combinations were tested. All seed boxes were covered with glass and incubated at 25° C. in a growth chamber. The most interesting combinations were those in which all layers were F. annosus or only B. bovinus, and in which F. annosus and B. bovinus were alternately layered. Seedlings in flats with F. annosus alone died rapidly. Norway spruce was more susceptible. However, only half the seedlings grown in the F. annosus and B. bovinus flats died, indicating a certain degree of protection to the seedlings by the symbiont. Mycorrhizae were not formed on their roots, therefore they cannot be implicated directly. However, indications were that an antibiotic was leached from the B. bovinus layer onto the lower layer of F. annosus which inhibited the latter's development. Similar results were obtained and conclusions made when another technique was used. This involved inoculating seedling roots growing with or without a layer of B. bovinus with F. annosus infested barley grains. An inhibitory substance was extracted from B. bovinus infested barley grains and separated by use of thin layer chromatography. The substance was not phytotoxic to spruce of pine seedlings. Research at our laboratory will continue in this area.

Marx: Kuhlman has recently found that conidia and basidiospores of F. annosus can survive in host-free non-sterile forest soil for as long as eleven months. He found that recovery of the pathogen sharply dropped to less than 50% after approximately two weeks but he was able to recover 5 to 10% after eleven months. He reported similar results regardless of soil type used. He varied soil pH and observed the same recovery trend between pH 4 and 7. Extremes of pH 3 and 8 caused a sharp reduction in recovery. He also investigated soil moisture influences and found F. annosus survived best in drier soil conditions. He found no major differences in the percent recovery of conidia or basidiospores and concluded that the spores did not germinate or persist as vegetative mycelium under these test conditions.

Marx: Available evidence suggests that (1) F. annosus does not exist as free-living mycelium in forest soils and therefore probably does not function in spread of the pathogen, (2) the inability of mycelium to grow in the soil is attributed to antagonism caused by various soil microorganisms

including, at least tentatively, ectotrophic mycorrhizal fungi and (3) conidia and basidiospores of F. annosus are able to exist for at least 11 months, probably as dormant propagules, in a variety of non-sterile forest soils.

DISCUSSION

Marx: Dr. Hüppel (1) did you attempt reisolation of F. annosus from those layers of inoculum in which Boletus bovinus apparently caused limited infection by this pathogen on spruce and pine seedlings? (2) What zone on the seedling roots were infected by F. annosus?

Hüppel: (1) Reisolation of F. annosus from these layers was attempted but were unsuccessful. We also used an aqueous extract of B. bovinus barley grain inoculum to irrigate seedlings growing in F. annosus infested soil and found that seedling survival was good and F. annosus was not recoverable. This again suggested a water soluble antibiotic produced by the mycorrhizal fungus which inhibited F. annosus. (2) The roots were infected at a very early age but the exact location was not determined. The seedlings apparently died due to post-emergence damping-off caused by F. annosus.

Malla: Dr. Hüppel, did F. annosus produce conidia on the barley grain inoculum or was it simply existing as vegetative mycelium?

Hüppel: Conidia of F. annosus were not produced on the inoculum but the mycelium did completely permeate the barley grains.

Shain: In regard to the competition of F. annosus with other microorganisms, how much work has been done to determine the influence of temperature on this interaction. It is my understanding that F. annosus can compete well with other organisms at lower temperatures.

Rishbeth: I believe that temperature is very important in these relationships and I think it is unfortunate that so much work has been done at 22-25° C. only. In England, the mean soil temperature, where these microbiological interactions occur, is about 10° C. and ranges from 5 to 15° C. We are badly in need of more information on this subject.

Hodges: How can one determine whether or not a forest stand will support rapid spread of F. annosus or no spread? Just because there is no infection does not mean that rapid spread would not take place. Until this can be determined there is no validity in assaying the soil microbiological populations between such areas.

Rishbeth: In Poland, certain soil types, especially abandoned farm lands, are particularly likely to promote serious damage by F. annosus which is predictable. In East Anglia a parallel situation exists on old farm lands where the soil is alkaline. The situation may be extreme for pine stands or within a stand since the soil situation may change within just a few meters. The boundary between the two situations is as sharp as that. We can predict that when stump infection occurs in certain of these soils types then killing of pines will almost automatically follow. In Calluna heath soils, however, very little attack develops. I agree with Dr. Hodges in that in certain areas of very uneven attack by F. annosus it is very difficult to be sure the plantation will remain healthy.

Yde-Andersen: In Denmark, we have Norway spruce growing on former arable land adjacent to stands on former woodland sites. Both stands had approximately the same percentage of stumps infected with F. annosus. However, on the former arable land trees have abundant butt rot and heavy mortality, whereas trees on the woodland sites will have much less butt rot and mortality. I think the F. annosus has not been able to spread as rapidly on the woodland site as it had on the other site.

Hodges: Is there evidence to suggest that F. annosus will grow epiphytically on small feeder roots, as used by Manka, or is the situation such that epiphytic growth takes place only on larger roots with well developed bark?

Marx: To examine Dominik's report that F. annosus would form ectotrophic mycorrhizae on conifer roots, we examined the symbiotic potential of this fungus with 5-month-old loblolly and shortleaf pine in aseptic cultures. Fomes annosus did not form mycorrhizae with either species but did induce abundant dichotomy of short roots on both pine species as do a variety of other soil fungi and bacteria. Histological examination revealed that F. annosus did not infect the small feeder roots but did develop a dense peritrophic or epiphytic mycelial habit on feeder root surfaces of both pine species.

Rishbeth: I do not believe that very much work has been done on the growth of F. annosus on very fine feeder roots of the type Manka described. In my experience, the further down soil samples are removed from soil profiles the less inhibition to mycelial growth of F. annosus occurs along roots. Samples removed from the litter and mycorrhizal layer are less likely to support growth over root surfaces. One point about Manka's work is that he sampled fungi from fine feeder roots located in the upper soil horizons rather than

a few inches down where the really critical transfers of mycelium occur on roots with bark scales, etc. The key to the problem, in my opinion, is studies of root surface effects at the lower depth in soil profiles of different soil types with a range of pine species. We may find a very different rhizosphere effect on roots at lower depths than on roots in the upper soil layers.

Houston: Dr. Marx, did I understand you to say that F. annosus did not infect the non-suberized feeder roots of pines grown aseptically?

Marx: That is correct, F. annosus existed only peritrophically. We did not however, histologically examine hardened-off 5 mm. or larger diameter roots of these seedlings for F. annosus infection.

Hodges: In regard to what Dr. Rishbeth said about studying microbiological population of the bark of roots larger than those used by Manka, we have found that old root bark of pine has a certain amount of nutritional value for F. annosus. In these pure culture studies, in which the bark was not nutritionally supplemented, we found a 3% weight loss after 6 weeks with F. annosus which is about 25% of what you find with wood. So it appears that F. annosus can indeed utilize bark as nutrients.

Malla: We have found that aqueous extracts (7.5%) of powdered 10-15 mm. diameter roots (including bark) of Norway spruce supplemented with 1% dextrose inhibited the growth of many soil fungi.

Rehfuess: We have found that bark from stems and roots of conifers stimulate the growth of many basidiomycetous and ascomycetous root destroying fungi (F. annosus not included). It takes a rather long time for the bark to be destroyed.

Burdekin: Will F. annosus grow epiphytically on the bark in advance of root infection of spruce as it does on bark of pine roots?

Yde-Andersen: In Denmark on former arable land, superficial growth of F. annosus mycelium is found in the bark of roots of Norway spruce. As far as I know, this growth habit does not occur on acid sites. This can perhaps be attributed to differences in population of microorganisms on root surfaces of trees in these different sites.

Rishbeth: We have found similar results in regard to F. annosus spread between former arable and acid heath sites in East Anglia. In our earlier work, we attributed these differences to a greater amount of Trichoderma viride present in the acid heath site than in the alkaline site.

Unfortunately we realize now that we were assaying only resting stages of T. viride and not its activity. Gibbs repeated this work and found that the T. viride group does not really differ greatly in population in the bark scales on roots of trees on the sites. It appears that different types of T. viride are operating in the different soil types. In addition, Gibbs found that different physiological types of F. annosus, varying with respect to sensitivity to antagonism by T. viride, exist in the different sites. Incidently another point I would like to make concerns methods used in antagonism studies. I think it better to use minimal nutrient media, such as plain agar, instead of potato-dextrose agar because results from rich media are totally unrepresentative of the effects you find in soil or roots.

Laatsch: Braun has shown by microscopic examination that F. annosus can penetrate roots of Norway spruce to the cork layer but not into the phloem. Cracks in the cork tissue will allow entry of F. annosus into the phloem tissue.

Marx: Can F. annosus penetrate a healthy living root of either spruce or pine with an active cambium? We know it exists on bark scales of roots under certain conditions but can it penetrate an intact and active cambium and cause an infection of phloem tissue?

Hodges: In acid soils of the United States, roots infected with F. annosus will have epiphytic growth of the fungus as far as several feet in advance of the main root infection. From this epiphytic habit, the fungus can infect at several locations along the root. In this way we may have several infections on a single root and not just a single infection of the root at a point of root contact with an infected stump.

Yde-Andersen: In Denmark, we have numerous examples in which healthy Norway spruce trees have been infected from adjacent infected stumps through root contacts and not root grafting. I cannot be sure, however, if wounds on the root were present or not.

Cowling: In the colonization of root bark are you speaking of a superficial mycelial growth entirely or do you find the fungus in the living tissue of the phloem as well?

Hodges: The fungus grows through the bark scales of pine above the cambium as invisible mycelial threads (detected by isolations) and is not in the living wood at all. At various points along the root we can find areas of resin exudation, a host response caused perhaps by some metabolic product of the fungus prior to its penetration of the xylem. Initially F. annosus

cannot be isolated from the xylem beneath these small resinous areas but it can be isolated at a later stage of infection. Eventually the root is girdled at these points and the fungus colonizes the root segments between the points of infection. Since these segments were dead or dying before colonization took place, no resin exudation occurs. Resin exudation and infiltration indicates the roots were alive at time of infection. The non-resinous areas decay at a more rapid rate than the resinous areas.

Rishbeth: Under East Anglian conditions, we can get resin exudation on pine roots due to wounding in the absence of *F. annosus*. I regard this reaction as a non-specific response caused by mechanical wounding, as in control inoculations with a sterile disc.

Hodges: Do you mean if you wound the root you get exudation zones ahead of the wound area?

Rishbeth: Yes, certainly. *F. annosus* in pine roots develops internally in the root and ephiphytically on the bark scales sometimes several feet in advance of the internal infection. Broadly speaking, when this infection stops it ceases both internally and epiphytically. However, in butt rot susceptible species such as larch, Norway and Sitka spruce we have a different situation. *F. annosus* may be stopped in the living bark and the outermost wood but it will proceed to advance in the middle wood and eventually enter the base of the tree. In this latter instance it is important to distinguish between the living bark and the dead bark scales. I think a very interesting point Dr. Hodges made was that of *F. annosus* in the bark scales of pine roots existed as invisible mycelial threads detectable only by isolation onto a specialized medium. This procedure was not available during our work in East Anglia when we concluded that *F. annosus* was more prevalent on roots in the alkaline soils than in acid soils in which it was not visible on the roots. Cultural methods now have shown it to be present on root bark scales in some acid sites, especially at depths below 15 cm.

Hodges: White pine in the southeastern United States reacts to *F. annosus* much the same as spruce. The fungus grows through the center of the root and not on the surface or near the cambium. When the fungus invades the butt of the tree through these roots isolated patches of decay are developed. Eventually these areas of decay spread several meters in the tree.

Marx: We have discussed a variety of organisms which are capable of antagonism against F. annosus. Does F. annosus have antagonistic potential?

Cowling: Bassett recently has isolated an antibiotic produced by F. annosus during its pre-autolytic phase of colony development. It is a very potent antibiotic capable of causing death in parenchyma cells of pine, Chlorella, and bacteria. It was not effective, however, against Trichoderma or Peniophora. We do not know the role of this antibiotic in the survival or persistance of F. annosus in soil.

Houston: Could the separate loci of infection by F. annosus along a root originate from soil inhabiting spores and not necessarily from a continuous mycelium sheath (however invisible) originating from the major root infection loci? Could a root exudate, perhaps originating at points of root breakage, stimulate these dormant spores to germinate and infect the root at these various points?

Rishbeth: I think it highly unlikely that this could occur because of soil fungistasis. However, we know very little about nutrient exudation from large roots of spruce or pine. This is a question that should be answered by experimentation.

Hodges: Kuhlman has recently found that as few as 40 conidia of F. annosus per ml. of water applied to intact roots of recently cut pine stumps (less than 1 month old) can cause infection. The soil was replaced over the inoculation as it existed originally. He finds a good correlation between conidial concentration and percent of root infection. In our opinion this could explain direct root infection in which F. annosus is found in roots where it could not possibly have entered through the cut stump surface or root grafts.

Laine: I think that we must investigate the possibility of insects such as Trypodendron, Hylastes, and Hylobius spp. carrying spores of F. annosus to healthy roots and through their feeding habits which cause injury and actually inoculate the root.

Punter: I have achieved about 15% infection of 3 cm. diameter stump roots by inoculating cut ends with 2000 viable basidiospores of F. annosus and replacing the root in the soil. Infection did not occur using conidia as inoculum. The roots were cut from treated stumps about 9 months old.

Hodges: Kuhlman found that more infection occurred when roots were inoculated on the horizonal surface rather than on cut ends.

Cowling: In Punter's experiment in which cut ends of roots were inoculated, the roots had the potential to secrete resin and the wood could undergo the dyamic changes reported by Shain. In contrast to Kuhlman's work on infection through intact bark, the tissue presumably is dead or at least has minimal activity. We have found that the nitrogen content of outer bark is much greater than that of the inner bark. This suggests that dead bark tissue high in nitrogen content is more favorable for germination of spores than is the more hostile environment of the internal root where both passive and dynamic responses to infection occur.

Dimitri: We also have studied the possibility of direct infection of roots by spores of F. annosus. Roots of living Norway spruce trees (60-70 years old and 40 cm. in diameter) were treated as follows: (1) alcohol wash only, (2) periderm removed (3) bark removed, and (4) outer sapwood removed in addition to bark. Fruit bodies of F. annosus were placed over each location for basidiospore inoculation. After inoculation all the roots were covered with plastic and the soil replaced. Genetic variation was eliminated by exposing roots of each tree to each treatment. The inoculations were performed on trees in different soil types and at different seasons of the year. Infection occurred only in roots in which the bark or the outer sapwood were removed. We concluded that basidiospores of F. annosus cannot infect intact roots of living trees but it can establish infection through wounds in the root.

Hodges: Is it possible that roots with thick bark, in which F. annosus may grow before penetration, may allow greater infection than roots with very thin bark containing less dead tissue?

Dimitri: The bark thickness of these Norway spruce roots was variable but averaged about 5 to 6 mm. We do not feel that bark thickness on spruce roots is a factor.

Laatsch: Braun found that F. annosus cannot infect roots of Norway spruce through the bark unless wounds are present. The fungus can penetrate some cork layers but not beyond the last cork layer containing flavopenes. The flavopenes, which are produced from phenols in the living bark, apparently were responsible for the lack of infection. Living bark of Norway spruce has approximately 20% of its weight as phenolic substances primarily in glucosidic form. Wounds in the living bark would allow F. annosus to bypass this chemical barrier. In addition, roots of stumps may not be as

resistant as are roots of standing trees because the stem and foliage are not furnishing carbohydrates necessary for constant production of the fungal inhibiting flavopenes or phenolic precursors.

Hüppel: We have performed inoculations of Norway spruce roots similar to Dimitri. We made deep wounds, superficial wounds and removed bark of roots and found that conidia of *F. annosus* would only infect roots having the deeper wounds. We also found that moisture content of wood in the deeper wounds were more favorable for optimal growth of *F. annosus*.

Liese: We have studied the possibility of direct root infection of 3-year-old Norway spruce seedlings in pots. Glass tubes were inserted vertically onto roots, fruit bodies of *F. annosus* were placed over the soil tube and the spores were leached into the soil tubes with water at intervals. Roots were removed after 3 months, cleaned with water and alcohol and incubated on sterile filter paper. *F. annosus* was not isolated from the roots. *Hypholoma fasciculare* was detected on the roots in most instances. The lack of root infection or recovery of *F. annosus* was probably due to antagonism by Hypholoma or bacteria in the soil column.

Rishbeth: I have found *Hypholoma fasciculare* to be antagonistic to *F. annosus*. Because of this characteristic, it was used as treatment for Norway and Sitka stumps in experiments. It had no effect on stump colonization by *F. annosus*. However, there is a great deal of difference between stump and root surfaces. It may have an influence on root surfaces that it may not have in a woody stump.

PANEL SESSION 4--PREDISPOSITION

W. Laatsch (Germany), Chairman, and K. E. Rehfuess (Germany)

INTRODUCTION

Dr. Laatsch first reviews investigations in Norway spruce stands of northwestern and southern Germany dealing with the relations between heart rot and site factors. He refers especially to results published by Rohmeder (1937), Zycha and Kató (1967) and to the papers of Laatsch and Rehfuess presented at this conference. Following this survey, he develops a working hypothesis on how the nutritional status of the trees may change the production of inhibitory substances or the mycorrhizal development and thus may influence the resistance of spruce against Fomes attack.

DISCUSSION

Zycha: In addition to the percentage of rotted trees, the ratio "diameter of the decayed area/diameter of the whole stem" in cross sections is a useful criterion to determine the degree of heart rot infection in a given stand. In spruce forests of northwestern Germany, this ratio seems to be a very characteristic feature specific for different site conditions, but independent of tree age, site class and percentage of rotted trees.

Shain: The described ratio is related to the time of infection as well as to the diameter of the heartwood. Spruce heartwood is known to be rather free

from inhibitory compounds. Therefore the decayed column in the stem should be able to invade the whole heartwood, given enough time. But when the infection reaches sapwood, further extension will be slowed down. Perhaps differences in nutrient supply on distinct sites govern the ratio heartwood/sapwood; this would explain the observations of Professor Zycha.

Zycha: That is possible, but we have not yet investigated this ratio in healthy trees.

Malla: The diameter of the decayed wood may be determined mainly by the time of infection.

Hodges: One can postulate that the time of infection will depend on the time of first thinning. That may be the reason why all the stands examined by Zycha get infected very early.

Rishbeth: If several spruce stands follow one another, you will indeed get infection at a very early stage possibly just after planting. Later thinnings may of course increase the amount of infections, but this does not influence very much spread of the infection.

Marx: Which site factor--if there is one--do you think to be the most important for this uniform early infection?

Laatsch: Several factors may operate, and they have a very strong effect, if they are working together. I already mentioned some of these factors: water shortage and lack of nitrogen, manganese, and potassium. All these deficiences decrease photosynthesis and the production of carbohydrates. On the other side, a surplus of nitrogen may be an important factor too, as one can conclude from the metabolic processes necessary to synthesize inhibitory substances.

Yde-Andersen: Recent investigations in about 450 spruce stands of Jutland heath areas have shown that the degree of butt rot attack is not related to the site class. But there exist loose correlations to pH and to the nutrient content of the humus layer. The degree of infection apparently decreases with increasing potassium and magnesium content of the soil, but rises with an increase of the manganese concentrations and in the soil pH. The higher the percentage of attacked trees, the higher is the proportion of heavily decayed stumps.

Roll-Hansen: Coming back to the question of Shain, I wonder whether an insufficient supply with nitrogen, manganese, and water causes a comparably narrow zone of sapwood. This effect could explain the

observation of Zycha that the ratio "diameter of the decayed area/diameter of the whole stem" is dependent on site conditions.

Laatsch: In relation to the interesting remarks of Yde-Andersen, the use of needle instead of soil analysis data probably would allow establishing much clearer relationships between the nutritional status of the stands and the degree of butt rot attack, because it is very difficult to determine the availability of the K-, Mg-, and Mn-amounts in the soil.

Yde-Andersen: If you compare the nutrient concentrations in the needles of healthy and of infected trees, you never know whether these levels are governed by the site conditions or by the infection itself.

Rehfuess: The needle samples of healthy and of attacked trees in the same stand are always analysed separately. Until now, with our sampling technique, we were not able to find any significant differences between these two groups.

Cowling: The nutrient levels in the needles only reflect the actual state of nutrition in the study year, which may be different from the nutrition at the time of infection, perhaps 50 years ago. Is not this a reason to be a bit cautious about foliage analysis, too?

Laatsch: Heart rot infection seems to affect neither the nutrition nor the growth of spruce stands. In southern Germany spruce stands, which show very good growth and which therefore must be well supplied with nutrients and water, can be heavily attacked. Only when the major part of the root system has been lost by decay does nutrient and water uptake decrease.

Cowling: Will you say that the potassium content of the foliage in a given stand on a given site, for instance, will not change year by year?

Rehfuess: Of course weather conditions induce variations of nutrient levels in a given stand from year to year, but the differences between the stands on different site units as a rule are not influenced. If you repeat the foliar analysis survey in another year, you will find the same ranging of site units. The element contents of the needles vary within a site-specific range.

Yde-Andersen: Are you talking only about Fomes rot or also about unspecified butt rot?

Laatsch: According to Zycha, Kató and Schönhar *Fomes annosus* is the most prominent of all butt rot fungi in spruce stands of western Germany. There

are other parasites too, but only the occurrence of Fomes annosus and the degree of heart rot induced by this fungus seem to be dependent on site conditions.

Rishbeth: It would be quite interesting to compare, on different sites, the development of heart rot infection in first rotation Norway spruce stands, where the timing of infection is known, even if this had to be achieved by stump inoculation or by direct tree inoculation.

Malla: How is it possible to determine exactly the start of infection in a given tree or in particular stands?

Zycha: There are no direct methods available. Therefore we tried it indirectly by comparing the number or percentage of infected trees with the age of the stands. These two features were not correlated.

Malla: Is it possible, that under certain circumstances infection starts very late?

Zycha: Theoretically yes.

Hodges: Is there any relationship between width and height of the rot column in the trees?

Zycha: Yes, there exists a positive correlation, but the variation is high.

Björkman: I am always interested to see if there are ecologically reasonable connections between different observations. In Sweden we have a severe Fomes attack both on very rich soils--on agricultural land or on calcareous soils where the growth of trees is excellent--and on the poorest sites, for instance on calluna heathland of the southeast. How can we understand this phenomenon? If you range Swedish forests according to the nitrogen and phosphorus content of their soils, you will find that the volume growth of these stands increases with increasing supply of nitrogen and phosphorus, whereas the root development decreases in the same direction. Mycorrhizal formation is good in the middle part, but low on both ends of this range, corresponding to the different amounts of soluble carbohydrates available in the trees. Conversely Fomes annosus attacks stands very strongly on the poorest and on the richest soils, but is of no great importance on medium sites. It is very interesting for me to hear that Professor Laatsch uses my carbohydrate-surplus-theory in order to explain the relationships between the degree of Fomes attack and the site

conditions. There is some evidence, that a surplus of carbohydrates in the roots promotes not only mycorrhizal formation, but also the production of inhibitory substances.

Marx: In southern pine forests of the USA, we generally find the same trend but not as strong as you. Our strongest effect of externally applying nutrients to the soil is not so much on the total number of mycorrhizae formed, but on which sort of symbionts will produce mycorrhizae under this set of nutritional conditions.

Björkman: Are the suppressed trees more severely infected by Fomes annosus than the dominant ones?

Laatsch: Zycha has found that the suppressed trees may be more heavily infected not by Fomes, but by other decay fungi.

Zycha: More recent investigations have shown also no difference in Fomes attack between dominant and suppressed trees.

Marx: How can you relate the degree of infection to the social position of the trees without knowing the social position at the time of infection?

Zycha: We only compared the percentage of infected trees among dominant and suppressed trees in given stands of today, and we couldn't find any significant differences between these groups.

Marx: What does this mean to your nutritional concept? Have you ever made foliar analysis comparisons between suppressed and dominant trees?

Rehfuess: At Tharandt near Dresden such comparisons were performed in spruce stands. They failed to reveal any clear differences in nutrient levels of predominant, dominant, codominant and dominated trees. Only the very suppressed trees had smaller needles, which seemed, in some cases at least, to contain less nitrogen and potassium than comparable needles of the other sociological classes.

Marx: What does this do to your photosynthetical concept?

Laatsch: The nutrient concentrations are derived as ratio between the element accumulation in the needles and the needle dry weight. These concentrations cannot reflect correctly the relations between the supply with growth factors on the one hand and photosynthesis efficiency on the other, if the minimum factor--in this case light intensity--is not taken into consideration. Under these conditions it is better to compare the amount of nutrient elements in a given number of needles.

Cowling: I am afraid I don't understand the relationship between lignification and resistance to Fomes, since the amount of lignin in spruce wood is a very constant figure around 27-28%.

Laatsch: I only wanted to demonstrate, that the same metabolic processes are involved in the formation of lignin as in the production of inhibitory substances. Trees oversupplied with nitrogen show insufficient lignification of the young shoots, which hang down like whips.

Cowling: Have you actually measured lignin content of these branches?

Laatsch: No.

Rishbeth: Is it possible, that the resin yield or perhaps the resin pressure would be increased in pines by nitrogen application?

Laatsch: We haven't yet determined resin production of pine forests. But if we fertilize N-deficient and slow growing pine stands with about 200 kg./ha. nitrogen, we observe, in the following year, a stronger resin secretion from the young shoots.

Björkman: In Sweden and Finland coniferous forests now are fertilized to an ever increasing extent. Many people complain that this procedure will tend to destroy the biological balance in forest soils and to promote Fomes attack. So far there is no real proof that normal fertilizing techniques in a practical scale will have these effects, but it is urgent to investigate this problem in a series of fertilizer trials.

Cowling: I cannot answer the question whether you destroy the soil or not. Neither can I answer the question if fertilizing does really predispose the trees for Fomes annosus. In our investigations in mature pine and spruce stands, where 130 and 200 kg./ha. urea had induced a fantastic increase of volume growth, we have never observed any change of the nitrogen content of wood or bark tissues. The fibre length, diameter of the individual cells, and the proportion of the parenchymatic tissue in the wood were likewise not altered. Therefore we don't anticipate any change in the decay resistance of the trees.

Laatsch: Perhaps you have applied just the physiologically optimal amount of nitrogen. But if you will increase the nitrogen supply more and more, and if you will repeat fertilizing year by year, you will get quite another situation and a remarkable accumulation of nitrogen in the soil.

Cowling: Don't you think that this is unlikely to happen for many decades, since the amount of nitrogen added is only a very small fraction of the total amount of nitrogen on the site?

Laatsch: The amount of fertilizer nitrogen is very high as compared with the amount of available soil nitrogen. Usually the mineralization rate of the nitrogen stored in the soil is less than 1% a year, and this mineralized nitrogen becomes available only slowly during the vegetation period. The urea nitrogen, on the contrary, is available in a few days. Furthermore we are fertilizing in Germany with 200 kg./ha. of N, which corresponds to more than 400 kg./ha. of urea.

Björkman: In Sweden too we give up to 200 kg./ha. of nitrogen. High urea amounts influence not only the pH and the microbiological situation in the soil, but also change the balance of the nutrients. An oversupply with urea nitrogen may lead to a lack of phosphorus or manganese.

Laatsch: Repeated fertilizing with nitrogen accelerates the breakdown of litter and raw humus. I think that the raw humus layers in coniferous forests help to maintain the balance between pathogenic fungi and their antagonists. Ploughing of clear cutted areas also leads to a rapid mineralization of the raw humus, and we know that Fomes attack in spruce stands or even in pine forests of northern Germany is very often more severe on ploughed soils than it is on unploughed forest land.

Rehfuess: Although N-fertilization had not changed the N-content of wood in the experiments described by Cowling, I suppose that there must have been a striking effect on the total amount of nitrogen accumulated in the wood, since the fertilization caused a distinct increase of volume growth and of dry matter production.

Cowling: That is correct, but all of our evidence points to the idea that it is not the absolute amount of nitrogen that influences the susceptibility to decay, but that it is the relative amount. The C/N-ratio of wood varies between 250:1 and 1250:1.

Rehfuess: The statistical relations between the nutrition and the percentage of rotted trees in our sample stands are rather difficult to explain and they don't allow drawing exact conclusions on the causal relationships between these features. It may be that there is an influence of nutrition via metabolism on the production of inhibitory substances or on mycorrhizal formation. On the other hand we have to consider that the nutritional status of these stands is mainly governed by the amount of

organic matter, by the pH and by the water regime in the soils. All these factors also will determine the composition and the activity of the microflora in the soil. Therefore we cannot exclude the fact that our correlations only reflect the variations of microbiological conditions in the soil.

Laatsch: What microbiological bioassay techniques are best to test the effect of inhibitory substances on Fomes annosus?

Marx and Shain recommend spore germination tests with conidia and basidiospores to be much more rapid than assay methods with vegetative mycelium growth.

PANEL SESSION 5--*FOMES ANNOSUS* IN SECOND-ROTATION STANDS

K. R. Shea (USA), Chairman, D. A. Burdekin (UK),
and A. Yde-Andersen (Denmark)

INTRODUCTION

Shea: The areas of discussion this morning concern *Fomes annosus* in second-rotation stands, its control by stump treatments, stump excavation, and delayed planting, and the economics of these treatments. Also, the potential importance of *F. annosus* in second-growth stands regenerated naturally or artificially by direct seeding or planting will be discussed. Papers by myself, Mr. Burdekin, and Dr. Yde-Andersen provide the background for this discussion section.

To open this section, I would like to give a brief resumé of *F. annosus* in the coastal regions of the northwestern United States. Forest practices involve converting old-growth, extensively managed forests to second-growth, intensively managed ones. Douglas-fir and western hemlock are the principal species under management. Root rots, principally caused by *Poria weirii*, *Armillaria mellea*, and *Fomes annosus*, are major causes of disease in these forests.

F. annosus is prevalent throughout the region primarily as a butt and trunk rot in western hemlock. Spore production is high over most of the year. Logging injuries on western hemlock and true firs (*Abies* spp.) are

highly susceptible to invasion by F. annosus, whereas injuries to Sitka spruce are seldom invaded. Recent research has shown that stumps of western hemlock are very susceptible to F. annosus but the question of spread from stumps to adjacent trees has not been fully explored.

DISCUSSION

Hodges: In your paper, you indicated that stump treatments in the Northwest frequently gave variable results.

Shea: Results with dry borax stump treatments suggest that other treatments may have to be developed that will be suitable under the high rainfall conditions of the region.

Hodges: In southern pines, copious resin production on the cut stump surfaces tend to retain borax and reduce the risk of loss from rainfall.

Shea: The susceptibility of logging injuries on western hemlock to F. annosus, especially those in contact with the soil, and perhaps of injured roots suggests that stump treatments alone may not be adequate to control the disease in stands not already infected, even if adequate stump treatments are found.

Houston: What is the effect of short rotations (40 to 60 years) on the future importance of annosus root rot in western hemlock in the Northwest?

Shea: The rapidity by which rot columns develop in injured hemlocks and the prevalence of butt rot in some young western hemlocks indicate that the problem will assume greater importance in future stands even with the shorter rotation ages.

Krstić: Do wounds in western hemlock appear more important as infection courts than roots?

Shea: At the present time, wounds appear more important in our area, but the role of roots, stumps, and injuries has not been fully assessed.

Shea: In western redcedar (Thuja plicata) susceptible to Fomes annosus?

Burdekin and others: Thuja plicata is highly susceptible to annosus heart rot in England and Ireland.

Robak: Why is Thuja plicata susceptible when an extractive, thujaplicin, is quite phytotoxic to F. annosus? Is thujaplicin found in living trees?

Cowling: Thujaplicin is found in living trees. It is, however, detoxified by some microorganisms; it also oxidizes to form less toxic dimers and

trimers. The interior (heart) of the tree is much less resistant to F. annosus than the outer portion (sapwood). F. annosus may have a more satisfactory means for detoxifying thujaplicin than other wood-destroying fungi.

Shea: Are artificially regenerated stands more susceptible to annosus root rot than natural ones?

Hodges: Natural stands in the southern United States appear to be more resistant to F. annosus than do plantations. In plantations in which large openings develop from F. annosus, natural regeneration frequently results. Over an 8-year period, it was rare to find a young natural seedling infected by F. annosus. Very dense stands frequently develop on old arable lands; but, even after thinning very little annosus root rot occurs as compared to planted stands on similar sites. It is difficult to artificially inoculate seedlings grown from seed in pots for approximately 1 year. Even when the seedlings are wounded, it is difficult to get infection. On the other hand, if pot-grown seedlings are transplanted to another pot and inoculated in the same way, 75 to 80 percent become infected. Similar experience occurred with seedlings obtained from a nursery.

Burdekin: Where does infection occur in the transplanted seedlings?

Hodges: Infection occurred at the point of wounding, near the root collar, and not on broken roots.

Rishbeth: Is the success of seedlings in annosus foci reflected in the rapid death of F. annosus in root systems once the hosts have been killed? Could the openings have been successfully regenerated by planting?

Hodges: We have tried to rationalize why 20 years after planting planted trees are more susceptible than natural ones but have not reached definite conclusions.

Marx: Is there a great difference in microbiological activities in soils between natural and planted stands?

Hodges: In the sandy soils of the southern United States, there appears to be little difference since litter disappears rapidly once a stand is open and very little litter accumulates. This may be unique to the southern United States, whereas in other portions of the world marked differences in soil microorganisms between plantations and natural stands may well occur.

Comments: Natural stands are much less likely to be severely damaged by annosus root rot than are planted ones. Regeneration obtained by seeding usually is not so severely affected as plantations.

Shea: At this time, I have asked Mr. Burdekin to discuss control of annosus root rot in Great Britain by stump treatments, stump excavation, and delayed plantings, and the economics of these control methods.

Burdekin: In many plantations where annosus root rot is not present, such as spruce plantations in Scotland and Wales, stump treatments can prevent Fomes annosus from becoming established. However, because of the widespread incidence of annosus root rot in many pine plantations in East Anglia, it is too late for stump treatments. Other methods are being attempted, viz, stump removal, delayed planting, and stump treatment with Peniophora.

Stump removal has been the most effective treatment. Mortality of young pines planted in treated sites was reduced from approximately 50 to 20 percent.

Delaying planting for 0, 2, 4, 6, 8, and 12 years also is being tried. Tentative results show a delay of 4 years reduces infection from about 50 percent to 35 percent with similar results for the 6-year delay. Delaying planting for 2 years was confounded by poor establishment and subsequent tree growth, so definite conclusion on the 2-year delay cannot be made. The 8 and 12-year delayed plantings have not been observed sufficiently long to draw conclusions. We do feel that one must delay planting at least for 4 years if this control method is used.

Hodges: As the plantation trees grow older, might not increased root contacts offset earlier results?

Burdekin: This could occur, but in the zero-years delayed plantings, now 12 years old, mortality is falling off. So far, mortality in delayed planting has resulted only from old stumps with no tree-to-tree spread from planted trees.

Stump treatments with Peniophora have been least successful of the three treatments so far. In theory, Peniophora grows rapidly and reduces the inoculum potential of Fomes annosus. In practice, the results have been variable.

Hodges: I have found Peniophora replaced Fomes annosus in newly inoculated wood, but wondered if the same were true for heavily decayed material.

Rishbeth: Root excavations have shown that Peniophora will not replace Fomes annosus present in stumps prior to felling and before treatment. Data suggest stump removal of the final crop may give the desired effect.

Burdekin: There was no difference in results when all old stumps were removed as compared with removal of those only from felled trees in one study; however, there had been only one earlier thinning prior to the final cut. When stumps of previous cuttings are at least 8 years old, one might have to remove only the stumps at the time of clear felling--about 300 stumps per acre.

Houston: Subsequent to stump removal, are special efforts made to avoid planting near old stump sites?

Burdekin: No particular care is taken to avoid a planting near an old stump site.

The distribution of losses from annosus root rot in plantations complicates greatly computations of economic impact. If losses were random, up to 40 percent of the trees could be killed; but since losses are not random, considerable financial losses result based on the distribution patterns found.

Rishbeth: In aerial photos of a plantation at 4 x 4 spacings, 30 percent loss at 10 years of age looked very serious, but at 25 years, the original openings could hardly be detected due to tree growth around the original foci. The situation is quite encouraging.

Burdekin: In our calculations, stump removal increased average revenues by 35 pounds per acre based on 50 percent losses in untreated check plots. Costs for stump removal were 20 pounds per acre, so stump removal increased revenues by 15 pounds per acre (all values are discounted at 3.5 percent).

Annosus root rot can be successfully attacked by several methods, including delayed planting and stump removal. Have others tried these two approaches?

Yde-Andersen: Old experiments in Denmark gave poor results, probably because the work was done by hand, leaving many infected roots. Current experiments on stump removal are still too new to yield results. Sodium nitrite is now being used as a stump treatment especially when the old stand is not heavily infected or with only a few foci.

Gremmen: Experiments on stump removal are being tried in the Netherlands but results are still pending.

Robak and others: Most practicing foresters would feel stump removal was too costly and would not consider it.

Burdekin: The reluctance to practice stump removal as a control is based on fears rather than on facts. Slowly this is being overcome where striking damage has occurred and something must be done if forests are to be grown successfully. Stumps can be removed in many ways and new and more efficient equipment could be developed to do the job more economically.

Kallio: There has been no stump removal in Finland so far.

Hüppel and Björkman: Uses for stumps can be found to improve the economics and perhaps foresters are too pessimistic regarding this control method.

Björkman: In Sweden, pine killing by annosus root rot is serious but the main problem is butt rot and decay in spruce. Investigations are planned to see if spruce can be replaced by other tree species. We are not especially hopeful for spruce in southwestern Sweden where it is the right tree for the site. We hope that utilization of partially decayed wood can be improved.

Cowling: Is consideration being given to control methods intermediate to stump removal and delayed planting. For example, chipping stumps.

Burdekin: So far, only stump removal has been tried, but deep plowing is being considered to break up the roots along with stump removal.

Shea: Is crop rotation using such crops as potato, lupine, and buckwheat as suggested in an early Danish pathology text being tried?

Yde-Andersen: Crop rotation gave very good results early in history, but this method has not been used for many years.

Shea: Have mixtures of conifers or conifers and hardwoods had any effects on annosus root rot?

Gremmen: In the Netherlands on poor soils, alder becomes infected and *Fomes annosus* spreads to other trees, including Scots pine.

Yde-Andersen: In Denmark in old woodland sites, mixtures of Norway spruce and beech resulted in heavy attack by *Armillaria mellea*. On heathlands in second-rotation stands, all broadleaf trees in the mixtures were killed by *Fomes annosus*.

Liese: Although there is no experimental evidence, spruce in south Germany does not seem so heavily attacked if mixed with beech.

Hüppel: There appears to be less *Fomes annosus* in mixed stands. About 5 years ago, large experimental areas were established in Sweden to test various mixtures.

Kallio and others: Annosus root rot is severe on Pinus sylvestris on sandy soils in Sweden. Fomes annosus also damages Alnus, Picea, and Betula. Pine is attacked only in the southern part of Sweden, never in the northern portion.

Hodges: Is there a relationship between potassium and annosus root rot since reports indicate potassium is considerably higher in wood decayed with Fomes annosus.

Comments: In the discussion that followed, the general opinion was that there is no relationship between potassium in the soil and growth of Fomes annosus.

Cowling: Mineral content in decayed wood is always higher, going up more than can be accounted for by changes in specific gravity. It may be possible that decayed wood becomes a metabolic sink in the tree.

Shea: In concluding this morning session, I want to thank each of you for your participation. We have noted considerable differences in host reaction to annosus root rot among species and within species. We can ask why. It is apparent we need to know much more about the importance of stumps, roots, and wounds as infection courts. We have reached the point in time when we can begin to study more fundamental aspects of the annosus root rot complex. Investigations on factors such as host-parasite interactions, fungus physiology, soil microbiology, rhizosphere phenomena, and on effects of various forest management practices can well provide keys for successful control of this disease.

PANEL SESSION 6--PHYSIOLOGY OF *FOMES ANNOSUS*

Ellis B. Cowling (USA), Chairman, and Martin Johansson (Sweden)

INTRODUCTION

The discussion began with a brief resume by Dr. Martin Johansson of the Royal College of Forestry, Stockholm, Sweden, of his contributed paper entitled: "Some aspects of metabolic studies of *Fomes annosus*".

DISCUSSION

Ricard: What is the minimum temperature for growth of *Fomes annosus*?

Cowling: At 5° C., a majority of 40 tissue isolates of *F. annosus* obtained from around the world grew at 10-15% of the rate of growth achieved at their optimum temperature--approximately 24° C. Preliminary experiments with certain of these isolates indicated that the fungus can grow at a degree or so below freezing.

Ricard: What is the minimum temperature for growth in wood?

Rishbeth: Eldon Ross in the United States has shown that the fungus will grow in wood at 5° C. I don't believe he has experimented with lower temperatures.

Ricard: Were these measurements made at constant or at fluctuating temperatures?

Rishbeth: Recent work with other fungi has shown that results obtained at constant temperatures provide a very realistic estimate of what happens at fluctuating temperatures.

Shea: Dr. Johansson, did you say that _F. annosus_ cannot use nitrate nitrogen?

Johansson: Yes, nitrate nitrogen is not satisfactory for growth of _F. annosus_.

Cowling: This is also true for other wood-destroying basidiomycetes including other root-rotting fungi such as _Poria weirii_ and _Armillaria mellea_.

Shain: Can _F. annosus_ carry on both aerobic and anaerobic respiration?

Johansson: Yes, both types of respiration have been demonstrated in this fungus, but it, like other fungi, cannot grow indefinitely without oxygen.

Shea: How do you think the fungus growing in the roots results in the death of trees?

Cowling: As yet we know very little about how _F. annosus_ kills trees. Most of what we do know about the physiology of this fungus relates to its capacity to cause decay rather than its capacity to kill the plant itself. I believe we should recognize that the capacity to cause decay may not be related to the capacity to cause mortality. This distinction is valid not only in comparing the effects of _F. annosus_ on spruce compared to pine but also in considering what physiological function is disrupted when infection leads to death of pines.

Some investigators have assumed that death of the tree is caused by decay of the roots. So far as we know, _F. annosus_ attacks the larger structural portion of roots, it does not attack feeder roots as in the case of _Phytophthora cinnamomi_. The function of the structural portions of the roots is primarily mechanical support and translocation of mineral nutrients and water upward and elaborated food materials downward. When infected trees are blown over by wind, certainly decay of the roots led to the death of the tree. But when a tree dies while standing it is not certain what caused it to die. If decay of roots led to a gradually decreasing capacity for conduction, one would expect to see a gradually decreasing rate of growth of the trees prior to their death. In the eastern United States and, I believe in other regions and countries as well, the last annual rings formed by pines prior to death by _F. annosus_ frequently are about the same

thickness as those formed earlier in the life of the tree. This suggests that death may not be due to a gradually increasing failure of roots. It also suggests that the factors leading to death may act in a matter of weeks of months rather than over the years that are necessary for development of the extensive decay we observe so frequently in trees that are killed by F. annosus.

It is well known that trees frequently survive and grow with only a fraction of their root system intact. This fact has led some investigators to assume that death results when F. annosus has finally decreased the functional root system to a level below that essential for maintenance of the water- or nutrient-balance of the tree. This is a reasonable explanation of mortality but its reasonableness is based on the as yet unsupported assumption that inadequate conduction through roots is the cause of death. Until this assumption is verified experimentally, I believe we should continue to consider other theories of pathogenesis as well.

A related theory is that mortality results when F. annosus girdles the stem at the root collar. If girdling means interruption of translocation of food materials in the phloem, such as occurs in other tree diseases such as white pine blister rust, an increase in diameter growth above the girdle frequently is observed. To my knowledge such an abnormal increase has not been reported. If girdling means interruption of the flow of sap in the xylem, then death must be assumed to be due to deficiency of water or mineral elements. Thus, the girdling hypothesis also involves the unsupported assumption that death results from decreased or interrupted transport of food materials downward or water and mineral elements upward in the tree.

Still another theory of how F. annosus kills pine trees involves the assumption that death of the tree is due to the action of a toxin produced in the roots and translocated to other parts of the tree. This theory also involves certain assumptions for which evidence is not available. It is consistent, however, with certain symptoms.

Dr. Shain showed recently that parenchyma cells are killed some distance ahead of mycelium of F. annosus as it advances through naturally or artificially inoculated loblolly pines. This is the only direct evidence of a toxin being produced in diseased trees. As you know, however, Dr. Colin Bassett has recently isolated a toxin from cultures of F. annosus. The structure of the toxin has been determined and it has been given the trivial

name fomannosin. It is a novel sesquiterpene. The toxin was shown to be effective against Chlorella, some bacteria and, most significantly, against loblolly pine. One-year-old seedlings of this species could be killed by applying very small amounts of the toxin to superficial wounds on the stem of the seedlings. Dr. Bassett was not able to isolate this substance from these treated seedlings or from naturally infected trees, but his efforts to do so were not exhaustive.

Laatsch: It seems to me that the rapid death of the seedlings treated with fomannosin contrasts with the long time it takes F. annosus to kill trees in the field?

Cowling: It does take a long time for F. annosus to kill mature trees in the field. But Dr. George Kuhlman, working in Dr. Hodges' group in the United States, has shown that the fungus can cause rapid death of seedlings in a greenhouse. By his technique, small segments of beech stem wood are inoculated and incubated with F. annosus for about 3 months. These infected segments are then fastened over small wounds on the root collar of healthy seedlings. Many seedlings inoculated in this way died in only 4-10 days after the beech segments were attached. Death in four days is both evidence that rapid death is possible and that death may be due to a toxin. It is difficult to invision how mycelium of F. annosus growing from the beech block into the seedling could have caused the seedlings to die merely by girdling the stem in as short a time as 4 days. It is much more plausible to suggest that a toxin accumulated in the beech block might have been translocated into the seedling and thus caused death of the living cells of the seedling in this short period of time.

Laatsch: Would you not expect that a toxin like fomannosin, if it is produced in infected root tissue, to be produced more or less constantly during colonization of the root tissue so that mortality would result from toxin acting over a long period of time?

Cowling: At London, just before coming to Aarhus, I had an opportunity to discuss this question with Dr. Dennis Garrett. He sees production of fomannosin by cultures of F. annosus as possibly similar to the production of other antibiotics such as penicillin. Bassett found that fomannosin was produced only in older (presumably autolyzing) cultures. It is known from physiological studies of many antibiotic-producing fungi that only trace amounts of antibiotics are produced by cultures during vegetative growth. After some essential nutrient other than carbon is exhausted in

the medium, however, a phenomenon called "carbon dissimilation" is initiated in the cultures. Carbon dissimilation involves the continuing assimilation of carbon but a lack of ability, due to lack of some other essential element, to continue to metabolize the assimilated carbon in the usual ways. Under such conditions new metabolic products (antibiotics) are secreted by the cultures. By analogy, it is possible that F. annosus develops in the roots of trees without producing fomannosin until some essential nutrient becomes deficient in the decaying wood. It is very common for F. annosus to have caused extremely advanced decay in roots before a tree is killed. At such times it is conceivable that some essential nutrient has become deficient and carbon dissimilation could lead to the production of the antibiotic, fomannosin. If it is translocated rapidly in the transpiration stream, it is reasonable to expect that rapid death of the tree could result without slowing down the rate of growth of the tree prior to the onset of fomannosin synthesis. To test these various hypotheses about the mechanism of pathogensis we need more evidence about the influence of infection by F. annosus on various tissues and organs in infected trees.

Cowling: Has anyone here measured the influence of F. annosus on water transport?

Shain: I have noticed that the moisture content of infected sapwood was lower than sapwood that was not infected. But this was in dead trees rather than in trees that were infected but still living.

Houston: Would it not be better to measure transpiration of the whole plant directly rather than just the moisture content of the wood?

Cowling: I think so. If you discovered that transpiration does decrease you could then use moisture-content measurements to locate the point in the roots or stems where the blockage occurred.

Laatsch: How do the symptoms of seedlings killed with fomannosin compare with those killed by inoculation with F. annosus.

Cowling: George Kuhlman and Colin Bassett have only experimented with loblolly pine so far. Seedlings of this species that are killed after inoculation with F. annosus show a typical grey-green coloration of the needles. Those killed with fomannosin show this same color change. Seedlings inoculated with the fungus in the fall frequently show a characteristic drooping of needles. This is the same symptom shown by seedlings treated with fomannosin if the seedling is not unusually vigorous. The time

necessary for death after inoculation with the fungus varies with the vigor of the seedling. Similarly, the amount of fomannosin required to kill loblolly pine seedlings varies with the vigor of the seedlings.

Rishbeth: Is fomannosin soluble in water?

Cowling: We do not yet know the limits of its solubility in water.

Shain: It could be transported in the oleoresin even if it is not soluble in water.

Rishbeth: This is an extremely interesting story. It reminds me very much of the observations on fusaric acid in relation to fusarium wilt disease. One point I would like to clarify though. In British plantations I don't think we have seen this drooping symptom. Do you suppose the conditions of growth of pines in the southern United States are such that you would get this symptom there but not in Britain.

Cowling: I do not believe we have seen this drooping of needles in field-killed trees either. As I mentioned it does show up in seedlings that are inoculated artificially in the fall. I wonder if perhaps this may not be due to an excessive dose of toxin applied to the seedling or perhaps translocated from the beech stem segments. More work is needed with various concentrations of fomannosin.

Rishbeth: I have noticed that pines grown in the greenhouse have been extraordinarily difficult to inoculate and frequently show no symptoms at all. But if they were placed outdoors in flats symptoms develop more normally.

Roll-Hansen: I believe that water deficiency is part of the symptom syndrome in trees infected with *F. annosus*.

Cowling: Certainly water stress could be part of the total symptom syndrome of the disease. I only question whether it is the primary cause of mortality--in other words that we ought to think of annosus root rot as a *wilt disease*.

Laatsch: I would like to say something more about nutrient concentrations and root diseases. Dr. Yde-Andersen and I applied lime to heath plantations in very high concentrations and planted pines the next year. The amounts of lime were sufficient to induce magnesium deficiency in agricultural crops. We have seen in Germany that spruce also is susceptible to manganese deficiency. Under such conditions the resistance of the plants would become low and infection would develop. If you analyze those stems now, several

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years after infection, they may show no difference in manganese content at all. But that doesn't say anything. You must analyze them in the first year when they become infected.

Cowling: I believe it would be desirable to do such nutritional experiments with artificially inoculated seedlings so we know the time of infection. Let me also mention again the notion that Dr. Zycha discussed earlier. He believes that factors that influence infection are different from factors that influence colonization of the host tissue. I would add that factors that influence both infection and colonization may be different from those that influence mortality.

Rehfuess: Have you ever tried to produce fomannosin in wood of loblolly pine or litter or humus?

Shain: I don't recall for sure whether Dr. Bassett succeeded in getting fomannosin from such cultures but I know he tried to do so.

Cowling: There are many questions that it would be interesting to answer about fomannosin. Is it produced in wood? What culture conditions influence its production? Is it water soluble? Is it translocated? Is it translocated as such or in some other chemical form? What tissues does it affect? What does it do to foliage? Many of these questions can be answered more easily now that we have a dependable method for inoculation of seedlings.

Rishbeth: It is important to realize that girdling of individual roots can go on for a long time before the whole root collar is attacked. Girdling at the root collar may be a late stage in a continuing process. Let me also add another comment about water relations. Killing attacks by F. annosus occur more frequently on light-textured soils where water stress is greater than on heavier soils.

Cowling: You could interpret this correlation as a factor contributing to disease development or to an influence on defense mechanisms of the host. It would be interesting to distinguish between the two.

Shain: We have been working with F. annosus for many years now. I was wondering if anyone had carefully observed trees in the process of dying.

de Brit: We have made some observations of this type. The trees typically show very sparce foliage, a few roots usually are alive or at least not as severely decayed as most of the roots. Usually too, some of the tissue in the root collar seems to be alive.

Houston: I have seen many trees of white pine that were growing well in both height and diameter and which were sitting on roots that were so badly decayed that I could easily push the tree over by hand. Such an infection must have existed for years. If such a tree is placed in a container of water I know that even though it has no roots at all it will continue to take up water and to grow.

Cowling: I have seen trees such as Dr. Houston has described. The condition of such trees persuades me that death must have been triggered by some new aspect of the host-parasite relationship that has not been important during the long time that these roots were being decayed. I was interested to pick up a paper by a Russian investigator who measured the increase in lumen diameter in roots affected to various degrees by F. annosus. The diameter increased with increasing decay. This increase in diameter could make decayed roots even more effective than sound roots for conduction of water, at least in trees like white pine that produce little resin.

de Brit: In the beech segment experiment described earlier, to what extent were the segments decayed? If it were extensive, would they not have contained fomannosin?

Cowling: The segments were decayed for 3 months and were significantly lower in specific gravity than sound beech wood. We have not yet determined whether, and if so to what extent, fomannosin accumulates in these segments.

Rishbeth: I would just like to support Dr. Houston's comment about trees with very decayed roots being able to take up water from wet soil. If the soil remains saturated, I expect severely affected trees could continue to live for a long time. I think a tree in this condition must be in a metabolically weakened state. Uptake of mineral elements is an active process requiring metabolism of root cells. If the roots are so badly rotted, I should think their uptake of nutrients, as opposed to water, might be reduced significantly.

Cowling: Dr. Laatsch spoke earlier about defense reactions in spruce. Let us give some attention now to the physiology of defense reaction in pines, Dr. Shain.

Shain: As many of you know we have described a reaction zone formed at the margin of infection by F. annosus in sapwood of loblolly pine. This zone is formed in advance of the fungus. It contains both pinosylvins and large amounts of resin. We have used nitro-blue tetrozolium to locate living

parenchyma cells of the tree in relation to the advancing fungus cells. The cells of the wood are killed in advance of the fungus. As they die, they undergo processes that are very similar to those that occur during formation of heartwood--stored starch and fats disappear and pinosylvins accumulate in the tissue. Epithelial parenchyma cells around resin ducts die and as a result resin flows into nearby cells. The phenols and resin provide a barrier to advance of the fungus. But in time, the fungus is able to break down the substances and thus breach this barrier. We showed that colonization of sapwood in suppressed trees that were inoculated artificially was much faster than in more vigorous co-dominant trees in the same stand. In suppressed trees that died after inoculation, the fungus grew 9 times further than in suppressed trees that remained alive after inoculation. The fungus grew further through the wood near the pith than through the wood near the cambium. All three of these observations indicate that the vigor of the host and its parenchyma cells determines the effectiveness of the defense by the host against invasion by F. annosus. I recognize that other types of defense mechanisms may be involved--maybe we were lucky in finding the simple relationship with phenols and resin that are already known to be inhibitory to F. annosus.

Rehfuess: Why do you think the fungus grew more rapidly through the tissues of the trees that died than those that remained alive?

Shain: After the tree died the sapwood could not react to the infection. Thus, the fungus was not impeded in its progress through the wood by phenols and resin. There was no reaction zone evident in the trees that died.

Laatsch: Does size of tree matter in terms of how rapidly the fungus can grow through the oldest sapwood?

Shain: There was not enough variation among just the co-dominant or just the suppressed trees, as I mentioned.

Cowling: I should like to emphasize the dynamic nature of the interactions Dr. Shain has described. He has shown evidence for the production of a substance that diffuses ahead of the fungus. This substance causes death of parenchyma cells. Death of these cells leads to the production of substances that in turn inhibit the fungus. The fungus then alters these inhibitory substances. Thus biochemical competition between the tree and the fungus goes on continually at the margin of infection so long as the tree remains alive. Perhaps in the future we will better understand these

competitive reactions and thus can alter our management practices in ways that will increase the effectiveness of the defense reactions of the host.

Yde-Andersen: Could not the slow penetration of spruce sapwood (see prepared paper by Shain) be explained by the activity of bacteria that are sometimes present; *i.e.*, production of methane, etc.?

Shain: Reaction zone formation apparently occurs independently of bacteria, but this could reflect the culture media used. Further isolations with additional media are planned. High pH suggests bacterial activity, but a microaerophilic environment, which may exist in the reaction zone due to high moisture content, gases, etc. may not, *per se*, seriously impede *F. annosus* since Gundersen (1961) found that the fungus could grow under microaerophilic conditions. Resistance of sapwood to decay has sometimes been attributed to its high moisture content. If this were so, reaction zones with a low moisture content (40-60% of dry weight) should be penetrated to their boundary with sound sapwood, but this was never observed.

Rishbeth: Bega and Tarry (1966) reported that pine oleoresin did not inhibit *F. annosus* but actually stimulated growth and sporulation. How can this be explained in view of other evidence that oleoresin is inhibiting?

Shain: Probably by their bioassay technique. The antibiotic-disc assay they used is quite good for substances with high antibiotic activity, particularly if they are water soluble. Oleoresin fulfills neither of these conditions. I tested various extracts with this technique and obtained negative results whereas inhibition was detectable when other bioassay techniques were used. Furthermore, inhibitory substances may stimulate growth at concentrations below their inhibitory threshold.

Björkman: Presented his prepared paper entitled "What To Do When Everything Else Fails".

Cowling: Your data show very well that wood decayed by *F. annosus* can be used to prepare satisfactory pulp. Is it not true that this same generalization can be made for wood decayed by other white-rot fungi, but not for brown-rot fungi.

Björkman: That is true. When pulping studies have been made with wood decayed by other fungi, the strength of pulp prepared from white-rotted wood was not greatly different from that of sound wood. But this was not true for brown-rotted wood.

PANEL SESSION 7--INTERACTION OF FOMES ANNOSUS AND OTHER FUNGI IN STEMS

J. Ricard (USA), Chairman, and M. Krstić (Yugoslavia)

INTRODUCTION

Ricard: Much research has been reported on the interaction of *Fomes annosus* and other fungi in and around roots, yet little work has been completed so far on these same relationships in stems.

This may be due in part to an unfortunate oversimplification whereby any fungus in wood is considered harmful. This is reflected perhaps in the widespread use of mass change measurements in wood blocks as an index of decay without consideration for the kind of carbon constituent utilized by the decay organism. Wood is a complex substance and though all of its components may be used for one application or another, it is seldom essential to have all of its cellulose, lignin, hemicellulose and simple sugars present *in toto*.

We are fortunate to have with us today one of the more active and experienced researchers engaged in the utilization of a fungus, *Penicillium rubrum* to control biologically a wood pathogen: Professor M. Krstić.

Krstić: At one time the outlook for the application of biological control to tree pathogens seemed rather dim as I discussed in 1956 in the Botanical

Gazette. However this view should be modified considering more recent findings, particularly those of Professor Bier in Canada.

In my work, observations on the Austrian and ponderosa pines in Southern Europe and California respectively, stimulated my interest in biological control. Open wounds in these trees are frequently overgrown by microbial flora leading to protection from tree pathogens. This event seems to coincide with a low resin exudation and would suggest that some antagonistic fungi could be used to delay the entrance of F. annosus.

In deciduous trees, much more information is available. In beech for instance, protection with molds can prevent up to a point, infection by Fomes fomentarius. Fresh cut logs of beech can be protected against penetration of the wood decay fungi, Hypoxylon granulosum and Schizophyllum commune by application of Penicillium sp.

My work has centered on the control of Endothia parasitica which grows between bark and wood in chestnut trees. Growth of fungi can take place in any portion of these trees. A number of fungal isolates was able to stop or delay the progress of various organisms. Penicillium rubrum was found particularly active and used for laboratory and field tests. When inoculated at the bottom of an incision through the bark, P. rubrum would grow and could be reisolated. However if both Penicillium and Endothia were introduced as a mixed inoculant, no satisfactory results were obtained as the pathogen appears to grow faster than Penicillium. In open wounds, inoculation with P. rubrum resulted in a five-fold decrease in infection by pathogens when compared with untreated wounds.

These encouraging results led to the preparation of conidia in large numbers for field applications. Beech and spruce sawdust was found to be colonized readily by P. rubrum. The colonized sawdust was applied to chestnut coppices near infected chestnut zones. Final results will not be available for several years yet, but should provide some indication of the effectiveness of P. rubrum in preventing infection in naturally occurring wounds.

Crystals of the antibiotic were obtained. Study of their chemical structure is underway at Durham, North Carolina and on the West Coast of the United States; their effect on various skin disease organisms is being evaluated currently. The antibiotic compound was found active against various human disease dermatophytes in Belgrade and it may be used for the control of superficial skin infections in humans. Aqueous solutions of the

antibiotic compound showed fungicidal and fungistatic properties against Endothia, F. annosus, and a number of other wood inhabiting fungi. It appears that F. annosus is rather sensitive to many antibiotics that have not yet been tested. I think that this flora could be used also for delaying or controlling the spread of F. annosus.

Ricard: Thank you, Professor Krstić for a most interesting and encouraging report.

In Orgeon, a local strain of Scytalidium sp. was used for field trials once its lack of adverse effect on the wood was demonstrated (1968). For rapid inoculation of Douglas-fir poles, a technique was developed whereby darts made from birchwood and permeated with the Oregon Scytalidium sp. were forced in the substrate by a standard drive tool similar to those used in the construction of buildings. The darts were prepared by flooding sterile birch dowel sections approximately 10 cm. long and 0.75 cm. in diameter in a malt extract suspension containing hyphal fragments of Scytalidium sp. Incubation at room temperature lasted 3 months. The dowel sections were then dried by evaporation under vacuum below 50° C. and reinforced at one end with a stainless steel arrow tip. Each dart was then tested for activity against P. carbonica by aseptic removal of a wood chip and cross plating on malt extract with the wood decay fungus. The satisfactory darts were fired in the groundline zone of Douglas-fir poles containing the incipient decay stage of P. carbonica. Six months later, the poles were tested as described earlier (1966) to determine if the Oregon Scytalidium sp. had become established in the infected poles. The organism was reisolated from poles scattered through various climatic regions in Western Oregon. Details are available elsewhere (In press).

It should be emphasized that antagonistic activity varies greatly from one Scytalidium sp. to another and even one strain and the next. Particular care was taken in 1965 in Oregon to select a strain of exceptional activity. This strain has been deposited with the American Type Culture Collection. Normal antagonistic activity is associated with the release of a yellow water soluble pigment by the fungus.

DISCUSSION

Rishbeth: I would like to hear just a little bit about the inoculation of Norway spruce and about the recovery of Scytalidium sp. This is a rather crucial point: to what extent did it grow and in what period.

Hüppel: Experiments started in 1963-1964 for that purpose were unsuccessful, because an unsuitable *Scytalidium* sp. strain was used, according to Dr. Klingström's explanations. Current experiments performed with the Oregon strain showed that upon inoculation with a wood dowel last spring, *Scytalidium* sp. was recovered 25 cm. above and 15 cm. below the point of inoculation. This experiment was made in a single tree, a live Norway spruce infected with *Fomes annosus*.

Cowling: Does *Scytalidium* sp. produce blue stain in wood?

Ricard: It depends perhaps on the species involved. None was observed from the Oregon strain.

Hüppel: No real blue stain was found in Sweden either. Perhaps a slight yellow green discoloration.

Ricard: In Oregon, a change in coloration of the wood was observed to a slight reddish brown in Douglas-fir and a definite yellow in birch.

Cowling: Are chlamydospores produced in wood?

Ricard: Not to the extent that they are found on malt extract agar; just a few, if any.

Johansson: Experiments performed in our institute have shown that the antibiotic produced by some *Scytalidium* strains are very active against *Fomes annosus*. The formation of the substance is dependent on a factor that seems to be present in xylan-preparations. Very small amounts of xylan, 0.1 g./l., increases the production appreciably. The substance has been chromatographed on this layer (silica gel) after ethyl ether extraction at a low pH. It has a low molecular weight, is water soluble and heat stable, and gives a faint blue fluorescence at 366 mμ.

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